The indoor environment and childhood allergic diseases; the importance of allergens and microbial components

by

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I keep six honest serving men,
(they thought me all I knew).
Their names are what, why, and when
and how and where and who.

Rudyard Kipling

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Oslo, April 2010
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CONTENTS

1 Preface

1.1 Abbreviations

BHR	bronchial hyperresponsiveness
BO	bronchial obstruction
CD	cluster of differentiation
CI	confidence interval
DRS	dose-response-slope (by inhaled methacholine dose)
ECA	the Environment and Childhood Asthma Study
EAACI	the European Academy of Allergology and Clinical Immunology
ECHRS	the European Community Respiratory Health Survey
ELISA	enzyme-linked immunosorbent assay
EU	endotoxin unit
FEV_1	forced expiratory volume in 1 second
FEF_{50}	forced expiratory flow at 50% of forced vital capacity
GA^2LEN	the Global Allergy and Asthma European Network
GM	geometric mean
HDM	house dust mite
Ig	immunoglobulin
IRAS	Institute for Risk Assessment Sciences
ISAAC	the International Study on Asthma and Allergies in Childhood
LAL	Limulus Amebocyte Lysate
OR	odds ratio
PD_{20}	provocation dose of methacholine causing 20% fall in FEV_1
PIAMA	the Prevention and Incidence of Asthma and Mite Allergy Study
rBO	recurrent bronchial obstruction
SD	standard deviation
SPT	skin prick test
Th	T helper cells

1.2 Summary

Introduction

The observed rapid increase in asthma prevalence over the past decades in Western societies has been attributed to numerous factors, including increased awareness of the disease, altered lifestyle and activity patterns, and changes in indoor and outdoor environmental exposures. Modern houses with effective insulation have reduced the natural, passive air exchange with consequently increased exposure to indoor allergens and pollutants. In areas with low exposure to mites and cockroaches, exposure to cat and dog allergens are thought to be more important for susceptible individuals in relation to allergic disease.

The main aim of the present study was to improve our knowledge about the association of the indoor environment and the development of allergic disease in Norwegian children.

The specific research questions were:

1. Is pet exposure influenced by a family history of allergic disease?
2. Is exposure to furred pets associated with development of allergic diseases?
3. Does exposure to house dust mite and microbial components influence the development of allergic diseases?
4. Do boys and girls differ with respect to exposure and in exposure-disease associations?

Methods

The present thesis reports results from the prospective birth cohort *the Environment and Childhood Asthma Study* (ECA) established in Oslo in 1992, with 3754 infants enrolled. Starting at the maternity ward, extensive questionnaires were completed by the parents every six months for the first two years of the children's life. At 2 years of age, all parents were invited to submit house dust samples. At 10 years of age 1215 children were invited to participate in a follow-up study. These children had either had lung function measured at birth (n=802) and/or had participated in a nested case-control study at 2 years of age (n=562) (cases being children with recurrent bronchial obstruction during the two first years of life). Overall, 1019/1215 (84%) children attended the 10-year follow-up study with clinical examination, measurement of lung function, bronchial responsiveness, exercise tests, skin prick tests, extensive parental interview and dust sampled from the child's bed.

Results

Pet keeping at the child's birth was not associated with a family history of allergic disease. At the 10-year follow-up study, 24% of the parents failed to report the pet they had kept during the child's first year of life according to the prospective questionnaires. Furthermore, single parenthood, parental smoking, and having older siblings all significantly increased the likelihood of acquiring pets after birth, whereas high parental education levels increased the likelihood of pet avoidance. Overall, 319 parents reported pet avoidance due to allergic disease in the family or the wish to prevent allergic disease in the child. Recurrent or persistent bronchial obstruction early in life and doctor diagnosed asthma at 10 years of age did not prevent the families from acquiring furry pets. However, doctor diagnosed allergic rhinoconjunctivitis before the age of 10 years was a strong determinant for never having kept cats or dogs and for reporting pet avoidance.

For 260 eligible children with lung function measured at birth *and* house dust sampled at 2 years of age, the odds of current asthma at 10 years increased with 1.20 (95% CI: 1.01, 1.43) per 10 µg/g increase in cat allergen (Feld d1) levels at 2 years. At 10 years of age, 34.4% of the children were sensitized to at least one inhalant allergen, and 17.8% were sensitized to both seasonal and perennial allergens. The majority of the children sensitized to cat and dog allergens at 10 years of age had never kept cats or dogs at home (85% and 91%, respectively).

House dust mite (HDM) allergen, Der p1, was detected in only 12.3% of the 797 mattress dust samples taken at age 10 years. Nevertheless, the children who were sensitized to Der p1 (8.8%) had significantly higher Der p1 levels in their mattress dust compared to children who were not sensitized. Early life exposure to dog allergen (Can f1), ß(1,3)-glucans, and endotoxin did not modify the risk of allergic disease at 10 years of age.

The 10-year old girls were more likely to keep cats and rodents compared to boys, and girls were also exposed to higher cat and dog allergen levels from mattress dust, regardless of pet ownership. Current rhinitis at 10 years of age (25.0% of all the 1019 children in the follow-up) was most often associated with allergic sensitization (72.8%). The children with current rhinitis were also more likely to suffer from multiple allergy-related co-morbidities like asthma, atopic eczema, and conjunctivitis (87.0% with at least one and 42.9% with at least two co-morbidities). The odds of current rhinitis increased with 1.20 (95% CI: 1.01, 1.42) per 1 µg/g increase in HDM allergen level in mattress dust for girls, but not for boys.

Conclusions

1. The results presented in this thesis show that families with children with allergic rhinitis often avoided owing cats and dogs. On the other hand, a family history of allergic disease did not significantly decrease the likelihood of pet keeping at the child's birth. *Reported* pet avoidance appeared to be most common in families where the parents had high education, whereas pet removal was found to be uncommon in the present cohort.

2. Increased cat allergen levels in homes at 2 years of age increased the risk of asthma at 10 years of age, whereas no association was seen with allergic sensitization at the age of 10. Cat and dog allergen levels in mattress dust at 10 years of age were not associated with concurrent symptoms of rhinitis.

3. House dust mite allergen levels in homes in Oslo were generally low, but children sensitized to Der p1 had significantly higher levels of Der p1 in their mattress dust compared to those who were not sensitized. Endotoxin and ß(1,3)-glucan levels in house dust measured at 2 years of age did not modify the allergen or pet-related risk of allergic disease or lung-function at 10 years of age.

4. Girls were more likely to keep furry pets and were also exposed to higher cat and dog allergen levels at 10 years of age. However, in the multivariate analyses, levels of HDM allergen, but not cat or dog allergens, were associated with an increased risk of current rhinitis, but this was observed among the girls only.

1.3 List of papers

Paper I

Bertelsen RJ, Lødrup Carlsen KC, Granum B, Carlsen K-H, Håland G, Devulapalli CS, Munthe-Kaas MC, Mowinckel P, Løvik M. Do allergic families avoid keeping furry pets? *Indoor Air,* doi:10.1111/j.1600-0668.2009.00640.x

Paper II

Bertelsen RJ, Lødrup Carlsen KC, Carlsen K-H, Granum B, Doekes G, Håland G, Mowinckel P, Løvik M. Childhood asthma and early life exposure to indoor allergens, endotoxin and ß(1,3)-glucans. *Clinical & Experimental Allergy,* doi:10.1111/j.1365-2222.2009.03424.x

Paper III

Bertelsen RJ, Lødrup Carlsen KC, Carlsen K-H. Rhinitis in children; Co-morbidities and phenotypes. S*ubmitted*

Paper IV

Bertelsen RJ, Instanes C, Granum B, Hetland G, Lødrup Carlsen KC, Carlsen K-H, Mowinckel P, Løvik M. Gender differences in indoor allergen exposure and association with current rhinitis. *Submitted*

2 General introduction and background

The work in the present thesis was performed within the Environment and Childhood Asthma Study (ECA), which comprises a number of completed and ongoing subprojects. The present subproject has its focus on the possible role of certain indoor environmental factors on the development of allergic sensitization, rhinitis and asthma. The collections of information by questionnaires, clinical examinations and serum analyses, as well as the definitions of asthma and rhinitis were previously defined as part of the ECA study. The present subproject concerns the laboratory analysis of dust samples for common indoor allergens and microbial components, the relationship between exposure to these agents and health outcomes, considering the impact of personal, social and environmental factors. The laboratory and epidemiological analyses are the particular responsibilities of the author.

2.1 Allergic disease

2.1.1 Definitions

Allergic disease is a collective term which is often used to describe health outcomes associated with reactions to allergens such as atopic eczema, urticaria, wheezing, rhinitis, conjunctivitis, asthma, contact dermatitis and food allergies. However, most of these conditions may also be triggered by non-allergic factors like infections, exercise, irritants, climatic factors and emotional stress (Brunetti *et al.*, 2007; Carlsen and Carlsen, 2008; Fokkens, 2002).

A nomenclature proposal prepared by representatives of the European Academy of Allergology and Clinical Immunology (EAACI) has defined 'allergy' as "*a hypersensitivity reaction initiated by specific immunological mechanisms*" (Johansson *et al.*, 2004) and 'hypersensitivity' by "*objectively reproducible symptoms or signs, initiated by exposure to a defined stimulus at a dose tolerated by normal subjects*". 'Atopy' is presently defined as "*a personal or familiar tendency to produce IgE antibodies in response to low doses of allergens, usually proteins, and to develop typical symptoms such as asthma, rhinoconjunctivitis, and eczema/dermatitis*" (Johansson *et al.*, 2004). However, exposure to elevated immunoglobulin E (IgE) levels against a specific allergen does not necessarily lead to clinical symptoms, and allergens may cause symptoms in individuals without detectable IgE in serum (Bodtger *et al.*, 2006). In the present work, the main focus will be on exposure to indoor allergens and microbial components that *may* influence IgE-mediated hypersensitivity in susceptible individuals, with particular emphasis on asthma and rhinitis as

defined in the individual papers. In the present work allergic sensitization is defined by a positive allergy test and allergic disease when in combination with symptoms.

2.1.2 Specific IgE and allergic reactions

Host defense mechanisms consist of innate and adaptive immunity. Innate immunity mediates the initial protection against infections and foreign molecules entering the body, whereas adaptive immunity in response to the first exposure, develops more slowly and mediates the later and more effective defense specifically tailored to combat the intruder (Abbas and Lichtman, 2006). Furthermore, the adaptive immune response provides the immune system with the ability to remember and quickly recognize specific pathogens and molecules, to mount faster, stronger and a more efficient defense the next time the pathogen is encountered.

IgE antibodies are part of the specific immune system, and represent one of five immunoglobulin classes that have very different functional properties (IgD, IgM, IgG, IgA and IgE). Antibody production by B cells (differentiating into plasma cells) is largely regulated by T helper cells. The adaptive immune system may respond by different helper T cell responses, like Th1 and Th2 (Murphy *et al.*, 2008). They are each designed to eliminate different types of pathogens. The Th1-dominated immune response activates a *cell-mediated immunity* and also certain types of humoral (antibody-mediated) immunity characterized by activation of cytotoxic T cells and macrophages as well as production of opsonising IgG1 antibodies. The Th1 response is important in the defence against infections (intracellular and many extracellular pathogens), and also mediates delayed-type hypersensitivity reactions and causes organ-specific auto-immune diseases. The Th2-dominated immune response is characterized by a certain type of *humoral immunity* which stimulates antibody-mediated responses of particular immunoglobulin classes (IgE and IgG4) and is important in the defence against parasites, toxins and to some extent extracellular microbes, and also mediate classic (IgE-mediated) immediate-type allergic responses. Antibodies prevent infection by blocking the ability of microbes to bind to and infect the host cells (in particular IgA), by promoting phagocytosis and intracellular killing of microbes (opsonisation), and by complement activation which may directly kill the microbe. Antibodies also bind to microbial toxins and prevent them from damaging host cells. Immediate-type allergic reactions are caused by IgE-mediated mast cell activation and release of potent mediators like histamine, prostaglandins and leukotriens. Also basophils have IgE receptors on their cell surface and have similar functions as mast cells in allergic reactions. However, as mast cells have been more thoroughly described, the main focus will be on these cells in the remainder of the

chapter. The classic Th1/Th2 paradigm has recently been revised due to the identification of novel helper T cell-subsets like Th17 cells and different regulatory T cells. Th17 cells have a role in clearing specific types of infectious organisms (for example Klebsiella) and in the development of autoimmune diseases (Miossec *et al.*, 2009). Th17 has also been proposed as a new marker for classification of non-allergic types of asthma (Oboki *et al.*, 2008). T regulatory cells are important in preventing excessive reactions in the immune system by suppressing immune responses and inflammatory reactions (Ozdemir *et al.*, 2009).

The allergic reaction, like all immune reactions, result from two temporally distinct processes, the sensitization phase (allergen encounter with development of the immune response) and the challenge phase (subsequent allergen encounter by the sensitized individual) (figure 1). In the sensitization phase, allergens enter via the mucosal surface and the skin and are taken up by dendritic cells, processed and then presented to naïve T lymphocytes. If antigen specific T lymphocytes recognize the epitopes on the allergen through their receptors and the proper co-stimulatory factors are present, the T lymphocytes are stimulated to proliferate and differentiate into Th2 lymphocytes. Th2 lymphocytes release a number of cytokines including interleukin 4 (IL-4) and IL-13, both of which are critical to the production of IgE by B lymphocytes, and IL-5 which is important for the recruitment and activation of eosinophils (Owen, 2007). The B lymphocyte differentiates into either memory B cells or plasma cells. Plasma cells reside in the bone marrow and continue to produce IgE. The IgE molecules bind to the high-affinity IgE receptors on mast cells and basophile cells which then become sensitized.

Both IgE and mast cells are concentrated in the mucosal tissues, and IgE antibodies are among the first defense molecules that an allergen encounters. When allergen cross-binds specific IgE bound to the IgE receptors of mast cells, the mast cells become activated, which in turn leads to degranulation and release of allergic mediators. This causes an inflammatory response, the “early response” of the immediate reaction. The cytokines and mediators released in the early phase initiate the later response, as the site of inflammation becomes infiltrated with effector cells such as Th2 cells, eosinophils, and other cells, causing a second wave of reaction (“late phase reaction”) (Gould and Sutton, 2008). The allergic hypersensitivity reactions reveal itself in characteristic signs and symptoms in the different target organs of allergy, the skin (such as urticaria and eczema), the nose (rhinitis), the lungs (asthma) and the gut (food allergic reactions) (Gould and Sutton, 2008). Reactions may be local in the challenged organ, but can also become systemic and affect more than one organ (anaphylaxis).

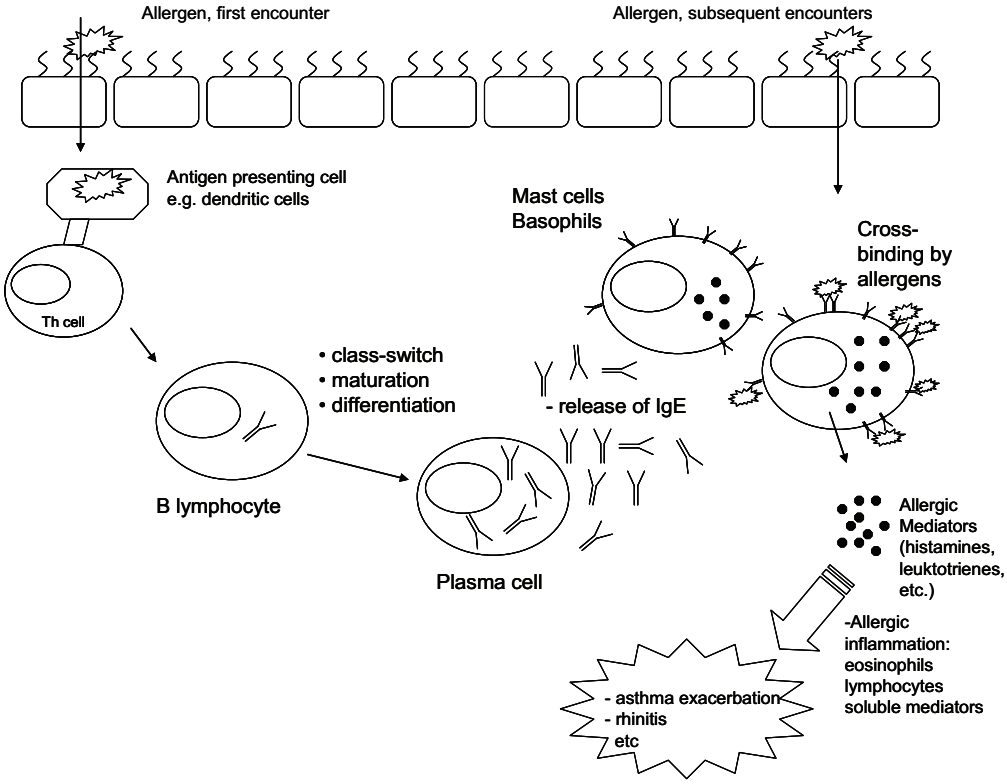

***Figure 1**: Schematic illustration of immunological mechanisms involved in development of allergic reactions*

Inhalation is a common route of allergen entry, and reactions to inhaled antigens with sneezing and a runny nose is often referred to as allergic *rhinitis.* Allergic rhinitis is characterized by intense itching and sneezing, local edema leading to blocked nasal passage, a clear nasal discharge, typically rich in eosinophils, and irritation of the nose as a result of histamine release (Murphy *et al.*, 2008). A similar reaction to airborne allergens deposited on the conjunctiva of the eyes is called allergic *conjunctivitis*.

Asthma is a chronic inflammatory airway disease characterized by bronchial hyperresponsiveness and reversible obstruction of airflow caused by contraction of the smooth muscles of the airways, oedema of the mucosa, excess mucus production, and chronic inflammation (Masoli *et al.*, 2004; Owen, 2007). The symptoms of bronchial asthma consist of recurrent episodes of wheezing, breathlessness and chest tightness which may cause considerable limitations and distress in the lives of those who suffer from this disease (Masoli *et al.*, 2004). However, it should be noted that all the above mentioned allergic diseases

(urticaria, rhinitis, conjunctivitis, asthma, and food allergy), may also be caused by non-allergic (non-IgE) mechanisms.

2.1.3 Diagnosis of allergic diseases and IgE detection

A variety of laboratory tests now exist to diagnose IgE-mediated conditions. Total IgE may indicate allergic inflammation, but may also show large variations between geographical areas and different populations (Burney *et al.*, 1997). Moreover, normal levels of total IgE does not exclude the presence of allergic disease (Sanz *et al.*, 1996). The most commonly used tests for determining the presence of allergen-specific IgE mediated inflammation are the skin prick tests (SPT) (Dreborg and Frew, 1993) and *in vitro* analysis for the presence and levels of specific IgE in serum (sIgE) (Tschopp *et al.*, 1998). SPT indirectly measures sIgE bound to mast cells/basophils in the skin where a single allergen molecule must simultaneously cross-bind two IgE molecules to cause a reaction. In an in vitro sIgE assay, the binding is monovalent and a single IgE molecule binds one epitope (antigenic binding site) to generate a reaction. Although IgE antibodies are produced as a response to the exposure from a specific allergen, cross-binding may occur in which the sIgE binds to other allergens with similar epitopes (Malandain, 2004; Reininger *et al.*, 2007). Cross-reactivity may thus have consequences for sIgE as diagnostic tools, and a careful patient's history in addition to the quantitative sIgE results is vital for diagnosing clinical allergy. A more important issue for the patient is that cross-binding to allergens with similar epitopes may lead to cross-allergies between food proteins, and also between food and pollen, the so-called oral allergy syndrome (Poulsen, 2005; Ricci *et al.*, 2005).

For both SPT and sIgE assays, more or less standardized allergen extracts are used, but the extracts may vary in potency from different manufacturers (Eichler *et al.*, 1988). Moreover, the allergen extracts may have only a selection of the relevant fractions or antigenic determinants (epitopes) which are responsible for the clinical symptoms. Thus, patients may experience allergic symptoms without a positive allergy test. The agreement between SPT and sIgE assays is in general good, but discrepancies may occur (Sanz *et al.*, 1996). Higher concentrations of sIgE and/or increasing SPT wheal size are roughly correlated with an higher probability of clinical allergy (Asarnoj *et al.*, 2008; Sicherer and Sampson, 2006).

Diagnosing allergic disease in children may be difficult because they are not always able to describe their symptoms (Lack, 2001). In asthma diagnosis, pulmonary function tests are important to document airflow limitations and are often used to confirm the presence of a

reversible bronchial obstruction after administration of a short-acting bronchodilator (Passalacqua and Ciprandi, 2008). However, such tests are difficult to perform in infants and young children who are unable to cooperate adequately. Thus, asthma in children younger than 6 years of age is commonly a clinical diagnosis, based on recurrent symptoms of airways obstruction such as cough, wheeze and shortness of breath. The presence of a family history of atopy, allergic sensitization and positive effects from asthma medication are used as additional indicators of asthma. However, in particular at pre-school age, symptoms such as sneezing, runny or blocked nose, as well as cough and wheezing may be caused by viral infections, which add to the difficulty of diagnosing asthma and rhinitis in young children (Eder *et al.*, 2006; Lack, 2001).

2.2 Personal risk factors

2.2.1 Age

In infants and young children, food allergy and atopic eczema are the most common allergic diseases (Sicherer and Sampson, 2006; Wuthrich, 1999). Sensitization to milk and egg allergens is usually outgrown, while peanut, tree nut, and seafood allergy often persist (Sicherer and Sampson, 2006). In later childhood, sensitization to inhalant allergens is more common than food sensitization (Hattevig *et al.*, 1993).

Atopic infants, however, are at risk of allergic diseases also later in life. It has been estimated that 35% of young children with atopic eczema have food allergy (Sicherer and Sampson, 2006), and this has further been proposed as the first step in a progressive “atopic march” that over time may lead to asthma and/or allergic rhinitis (Burgess *et al.*, 2009; Gustafsson *et al.*, 2000; Spergel and Paller, 2003). Wheezy infants with IgE antibodies to foods or inhalant allergens are reported to have increased risk of respiratory symptoms later in childhood (Kotaniemi-Syrjanen *et al.*, 2003). Rhinitis often precedes the development of asthma (Cruz *et al.*, 2007), but it is generally not clear whether one allergic disease leads to another allergic disease, or to what extent allergic sensitization is causative for or co-existing with rhinitis and asthma (Anto, 2004). Therefore, the term ‘atopic march’ should not be used to describe causal relationship, but rather the time sequence of disease manifestation. Nevertheless, diagnosing allergy in young children is important because it has prognostic implications and sets the basis for managements and preventive strategies (Sly *et al.*, 2008).

2.2.2 Gender

Boys and girls have different prevalence of asthma and allergy and appear also to differ in their response to the triggers of these diseases. Boys are consistently reported to have more prevalent asthma and allergies than girls before puberty. In adolescence, the pattern changes and a higher prevalence of asthma and allergies are found in females (Almqvist *et al.*, 2008; Postma, 2007). Females appear to be under-diagnosed with regard to airway diseases, however, this has been found both in childhood and adulthood (Siersted *et al.*, 1998; Yeatts *et al.*, 2003) and is therefore not likely to explain the gender differences. No single straightforward mechanism can explain the gender differences in allergic disease although extensive research has been focused on this topic. The differences are thus more likely to be a combination of physiology (Becklake and Kauffmann, 1999; Lowe *et al.*, 2008), differences in susceptibility of environmental factors (Gold *et al.*, 1996; Lodrup Carlsen *et al.*, 2006b), and differences in risk factors, such as smoking and pet keeping (Bjerg *et al.*, 2009; Chen *et al.*, 2002).

2.2.3 Genes

The factors, which determine whether an allergen will trigger a Th1 or Th2 response, are not well understood, but as implied by the definition of atopy, allergic diseases have an important hereditary nature. For example, the risk of a child developing an IgE-mediated allergy is 40-60% if both parents are allergic, and 5-10% if neither parent have allergy (Kjellman, 1977). This, however, have changed over the last decades and the risk for developing allergic disease for individuals without a hereditary predisposition is now believed to be much higher than 5-10%. Parental asthma is also a risk factor for the development of early allergic sensitization in children (Crestani *et al.*, 2004). For asthma, twin studies have demonstrated a considerable genetic component, but the genetic contribution differs for the different phenotypes of asthma (Los *et al.*, 2001). Moreover, environmental exposure affecting individuals differently depending on their genotype, by so-called gene-by-environment interactions (Bruce *et al.*, 2009; Eder *et al.*, 2004), are becoming clearly more evident. A model for such interactions is suggested by the endotoxin-CD14/Toll-like receptor interaction, indicating that the protective or detrimental effects exerted by specified genotypes on allergy or asthma depend upon the exposure levels (Eder *et al.*, 2004). The importance of environmental factors are supported by numerous epidemiological studies showing major differences between populations that are not related to genetics alone, such as populations in Hong Kong *vs* other Chinese cities (The

International Study of Asthma and Allergies in Childhood (ISAAC) Steering Committee, 1998; Wong *et al.*, 2001) and populations in East *vs* West Germany (Ring *et al.*, 1999).

2.3 Environmental risk factors

2.3.1 Geographical trends

Differences in climate, location (urban, rural), cultural and other life style factors all lead to different types of exposure that may have consequences for the development of allergic disease. The severity and high prevalence of asthma (>20%) observed in Australia, New Zealand, Taiwan, the UK and inner cities in the US, are proposed to be related to mite and cockroach exposure (Richardson *et al.*, 2005) as well as to socio-economic factors. However, in other countries such as Sweden and Norway and in high-altitude locations where these potent allergens are absent, or present in very low concentrations, the prevalence of asthma is still high (Lodrup Carlsen *et al.*, 2006a; Perzanowski *et al.*, 1999; Sporik *et al.*, 1995). In these regions, exposure to cat and dog allergens is hypothesized to be more strongly linked to asthma (Custovic *et al.*, 1998; Perzanowski *et al.*, 1999).

Although sensitization to cat and dog allergens usually is more prevalent than to mite and cockroach in Scandinavian countries, pollen is often the dominant sensitizing allergen, in particular birch, due to relatively short periods of high birch pollen exposure (Bakken *et al.*, 2007; Omenaas *et al.*, 1994). In Sweden, birch pollen was found to be the dominating inhalant allergen as early as 4 years of age (Asarnoj *et al.*, 2008), and in the ISAAC study, Norwegian children had the highest prevalence of allergic sensitization to seasonal allergens among all the included populations (Weinmayr *et al.*, 2008). The prevalence of allergic sensitization in asthmatics may also vary depending on geographical location, for example from 56.3% in 10 year old children in Oslo (Lodrup Carlsen *et al.*, 2006a) to 70.1% in Tuscon (Crestani *et al.*, 2004). The allergens needed to be included in test profiles to help identifying individuals with allergic disease, will vary depending on the subject's place of residence as well as age (Wickman *et al.*, 2005). In a recent Global Allergy and Asthma European Network (GA^2LEN) study, as many as 18 different allergens had to be included in a skin test panel to appropriately assess sensitization across Europe (Bousquet *et al.*, 2009).

2.3.2 Time trends

The increase in allergic diseases has often been referred to as the Epidemic of the 21st Century. Asthma showed a marked increase in the decades between 1960 and 2000, in particular in affluent countries. Over the past decade, however, asthma prevalence seems to

have levelled out or even declined in some countries (Asher *et al.*, 2006). A recent study from our study group in Oslo, Norway, demonstrated a life-time prevalence of asthma of 20.2% among 10 year old urban school children and current asthma in 11.1% (Lodrup Carlsen *et al.*, 2006a). This is the highest prevalence reported in Norway and shows an exponentially increasing trend from 0.4% in 1948 (Claussen, 1948).

For allergic rhinoconjunctivitis, the pattern is complex, depicting a scenario where all three possibilities of increasing, decreasing or steady trends are present in various regions of the world (Asher *et al.*, 2006). However, correct estimates of rhinoconjunctivitis in childhood are hampered by the lack of good age-specific diagnostic criteria and examinations, and the disease is usually defined by questionnaire data in which misclassification may occur (Phipatanakul, 2005), in that symptoms of rhinitis may be confused with symptoms of infections and colds. Problems may also occur in studies using objective methods such as skin prick test or allergen-specific IgE analyses as 'positive' tests indicate allergic sensitization, but not necessarily clinical disease (Phipatanakul, 2005). It has also been difficult to monitor trends in allergic sensitization, but it is generally accepted that the increase in asthma prevalence has been associated with a rise in allergic sensitization in the western societies (Braman, 2006). Furthermore, the prevalence of allergic sensitization has been found to increase in parallel in asthmatic and non-asthmatic subjects (Ronchetti *et al.*, 2007). A recent study from northern Sweden reported a major increase in allergic sensitization in school children from 1996 to 2006 by comparing two population-based cohorts from the same Swedish towns established 10 years apart (Ronmark *et al.*, 2009). The increase in allergic disease in many developed countries has been too rapid to account for corresponding changes in genetic susceptibility of the populations. It therefore seems likely that changes in environmental factors have had influence on the development of disease (von Mutius, 2000).

2.3.3 Social trends and lifestyle factors

In 1873 Charles Blackley observed that "catarrhus aestivus" (hay fever) was more common among the educated than the illiterate and he hypothesized that "as civilisation and education advance, the disorder will become more common than it is at the present time" (Blackley C.H., 1988). Still, more than a decade later, atopic diseases and allergic sensitization appear to be more frequent in the higher socio-economic classes within a community (Heinrich *et al.*, 1998; Williams *et al.*, 1994). In contrast, more severe asthma (Volmer, 2001) and non-atopic conditions are more common in the lower socio-economic classes (Ellison-Loschmann *et al.*, 2007). Within the U.S., social trends have in part been attributed to non-compliance to

treatment and reduced access to healthcare services for the lower social classes (Volmer, 2001). Other potential explanatory factors for differences between social classes may be differences in allergen types or lifestyle factors such as reduced physical activity, obesity, respiratory infections in childhood, smoking and exposure to tobacco smoke (Kitch *et al.*, 2000; Lannero *et al.*, 2002).

Social trends may also differ between countries and in Norway with government-funded education and healthcare; there are no clear distinct social classes. Furthermore, asthmatic 13 year old adolescents from the present cohort were neither less physically active nor more obese than the non-asthmatics (Berntsen *et al.*, 2009). Moreover, in two studies from Sweden, there were no clear socio-economic trend for allergic disease (Almqvist *et al.*, 2005; Braback *et al.*, 2005), and the steepest increase in asthma and allergic rhinitis was observed in the lower socio-economic classes (Braback *et al.*, 2005). In recent reports on world-wide trends on asthma the asthma prevalence appear to continue to rise sharply in developing regions with increasing urbanization and westernization (Asher *et al.*, 2006; Braman, 2006). The prevalence rates of allergic disease may also depend upon increased awareness of symptoms and from the willingness of physicians to make the diagnosis and prescribe treatment (Barraclough *et al.*, 2002; Eder *et al.*, 2006; Phipatanakul, 2005). However, despite a different labeling of asthma, a comparative study between three countries (Sweden, Finland and Estonia) demonstrated similar frequency of symptoms in the study populations (Pallasaho *et al.*, 2005).

Because the affluent countries have witnessed the highest increase in asthma and allergies during the last decades, environmental and lifestyle factors associated with the 'modern' or 'western' society have been suggested to influence the development of allergic disease (Von Hertzen and Haahtela, 2004). This has been supported by epidemiological studies reporting similar asthma prevalence trends between immigrant and non-immigrant children within the same area (Ponsonby *et al.*, 2008), and increased prevalence of allergic disease among the children from East Germany after the fall of the Berlin wall (Heinrich *et al.*, 2002). A similar East-West trend for asthma was observed in the ISAAC study for countries within Europe in 1998 (The International Study of Asthma and Allergies in Childhood (ISAAC) Steering Committee, 1998), whereas in 2003 this trend was clearly reduced (Asher *et al.*, 2006). Some of the proposed explanatory factors are changes in the microbial burden "The Hygiene Hypothesis" (Strachan, 2000), the occupational environment (Mapp *et al.*, 2005), a more sedentary lifestyle with changes in diet and physical activity (Alfredo *et al.*, 2007; Flaherman and Rutherford, 2006; Marti *et al.*, 2004), and increases in

traffic-related air pollutants (Braback and Forsberg, 2009; Gilmour *et al.*, 2006; Heinrich and Wichmann, 2004).

Air pollutants may act both as adjuvants or triggers of allergic disease (figure 2), and may together with climatic changes increase the abundance and effect of aeroallergens such as pollen (Gilmour *et al.*, 2006). Air pollutants in the indoor environment, such as tobacco smoke has been *consistently* linked to the onset of asthma (Carlsen and Carlsen, 2008; Eder *et al.*, 2006; Gilmour *et al.*, 2006). Lately, there has been a growing concern related to the increased exposure to industrially produced chemicals such as bisphenol A and phthalates (Lakind and Naiman, 2008; Wittassek *et al.*, 2007) that have been proposed to influence the development of allergic disease (Glue *et al.*, 2005; Tian *et al.*, 2003).

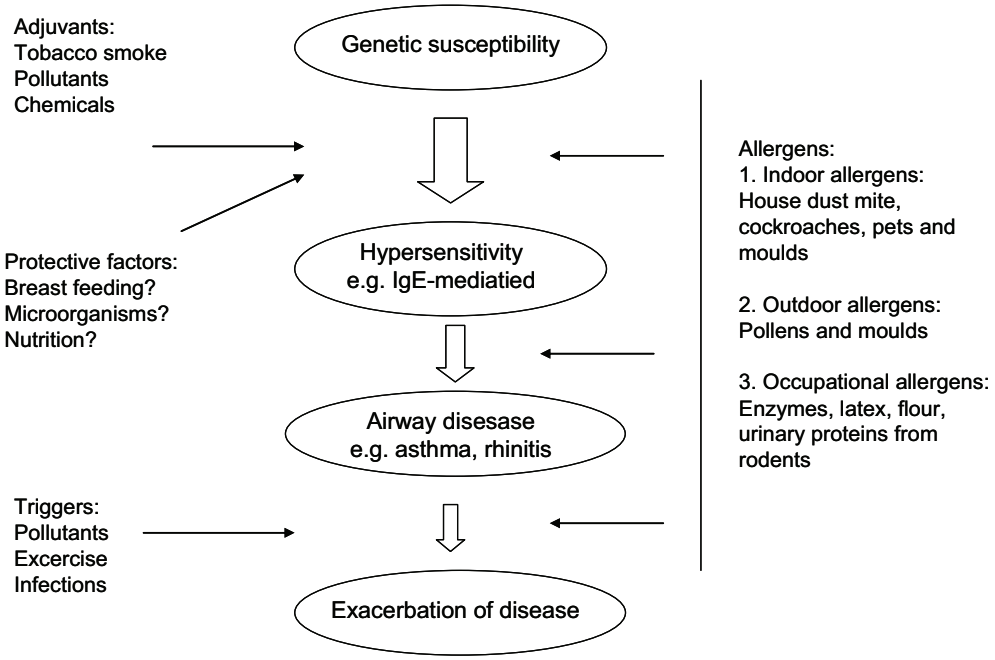

Figure 2: *Factors affecting the development and progression of allergic airway disease. Modified from (Nielsen G.D. et al., 2005)*

2.3.4 The indoor environment

Today children spend more time indoors and are less physically active than for some decades ago. It is estimated that the Americans spend an average of 87% of their time indoors (Klepeis *et al.*, 2001), and in a study from Germany the mean average time the individuals spent indoors in their *own home* was 16 hours (Brasche and Bischof, 2005). The housing

characteristics have also changed during the last decades (Jacobs *et al.*, 2009). Modern houses are better insulated which may cause an increase in indoor humidity and temperature. Increased dampness seems to be well documented and to be associated with airway symptoms and asthma, although the mechanism is not known (Bornehag *et al.*, 2001; Bornehag *et al.*, 2004; Gilmour *et al.*, 2006). Better insulation also leads to a dramatic reduction of the natural outdoor-indoor air exchange (Bornehag *et al.*, 2005). Under these conditions, allergens and pollutants produced or present indoors will no be diluted by natural air circulation, with the consequence of an increase in exposure.

2.3.5 Pet keeping and indoor allergens

The risk of developing **allergic sensitization** may be affected by the exposure level to indoor allergens. The dose-response relationship between allergen exposure and allergic sensitization may differ between allergens. A linear relationship for HDM and cockroach allergen exposure has been suggested, whereas for cat allergen exposure a bell-shaped curve leading to some form of immunological deviation and tolerance at the highest exposure levels has been proposed (Platts-Mills *et al.*, 2004). However, a non-linear relationship between HDM allergen exposure (mean exposure over a five-year period) and HDM sensitization and asthma at 5 years of age, was recently reported from a birth cohort/intervention study in Australia (Tovey *et al.*, 2008).

In prospective cohort studies positive associations between HDM allergen exposure and sensitization has only been observed for high-risk children (children with parental history of allergic disease) (Celedon *et al.*, 2007; Cole *et al.*, 2004; Sporik *et al.*, 1990) or in atopy-enriched cohorts (Brussee *et al.*, 2005; Wahn *et al.*, 1997). For population-based studies the associations are weaker, but interestingly, the associations between HDM exposure and sensitization appear to rise steeply at low levels and levelling of at high levels of exposure (Cullinan *et al.*, 2004; Torrent *et al.*, 2006).

With regard to pet allergens, similar associations as for HDM allergens have been seen in population-based studies for low levels of allergen exposure and sensitization, but with a lack of associations at high levels (Cullinan *et al.*, 2004; Torrent *et al.*, 2006). Moreover, several studies have reported exposure to cat allergens early in life to increases the risk of allergic sensitization to cat in children younger than 4 years of age (Brussee *et al.*, 2005; Chen *et al.*, 2007; Sandin *et al.*, 2004; Torrent *et al.*, 2006; Wahn *et al.*, 1997). In contrast, no association has been confirmed between dog allergen exposure and sensitization to dog (Brussee *et al.*, 2005).

Several studies have reported impaired lung function in individuals sensitized *and* concomitant exposed to high levels of the sensitizing allergen (Illi *et al.*, 2006; Lowe *et al.*, 2004) and allergic sensitization has repeatedly been found to be a risk factor for **asthma** (Illi *et al.*, 2006; Ingram *et al.*, 1995; Lau *et al.*, 2005; Platts-Mills *et al.*, 2001). However, when the association between allergen exposure and asthma development are assessed without the inclusion of allergic sensitization, usually no association have been confirmed. One review article reported that no longitudinal studies of random populations published before the year 2000 had been identified in which allergen exposure during infancy could be related to asthma risk after the age of 6 years (Pearce *et al.*, 2000). The majority of the later publications have also been unable to demonstrate an association between early life exposure to allergens and the development of asthma (Brussee *et al.*, 2005; Cullinan *et al.*, 2004; Lau *et al.*, 2000). However, one recent study reported increased risk of asthma in 6-year-old children exposed to cat allergens early in life, but no association was seen for mite allergen (Torrent *et al.*, 2007). On the other hand, early-life exposure to high mite allergen levels appears to increase the risk of asthma development in high-risk children (Almqvist *et al.*, 2003; Brussee *et al.*, 2005; Celedon *et al.*, 2007).

Exposure to pet and HDM allergens may also increase the risk of **rhinitis**. However, although rhinitis and asthma often co-exist, and may be attributed to a common airways disease starting at the nose and including asthma (Lack, 2001; Leynaert *et al.*, 2004), indoor allergens may exacerbate symptoms and increase BHR also in non-asthmatics with allergic rhinitis (Ciprandi *et al.*, 2005). Moreover, even low allergen levels may induce symptoms in sensitized patients, as was shown by demonstrating nasal symptoms in patients challenged with concentrations of cat allergen (Fel d1) comparable to allergen levels in homes without cats (Bollinger *et al.*, 1996). However, low allergen levels may also induce rhinitis symptoms in patients without detectable systemic IgE (Huggins and Brostoff, 1975)

Other indoor allergens than cat, mite and dog allergens, which are usually included in the allergy test panels are mould and cockroach allergens. Although sensitization to airborne moulds has been related to the severity of asthma (Zureik *et al.*, 2002), the link between indoor mould exposure as a risk factor for asthma development remains uncertain (Douwes and Pearce, 2003). Indoor mould exposure is uncommon in Norway, but for the few individuals that are exposed the effect on respiratory symptoms may be severe (Skorge *et al.*, 2005). Exposure to cockroach allergens has been found to exacerbate asthma in children living in the inner-city (Rosenstreich *et al.*, 1997). In Norway, cockroaches are uncommon,

but sensitization to cockroach allergens has been found among the children in a family study on asthma genetics (Lodrup Carlsen *et al.*, 2002).

In contrast to HDM exposure that occurs in all habitats with a suitable environment, a susceptible individual may choose to try to avoid cat and dog allergen exposure by not keeping pets at home. Moreover, studies assessing pet keeping *per se*, rather than pet allergen levels, have reported pet keeping to increase, decrease or to have no effect on the risk of asthma development (Almqvist *et al.*, 2003; Kerkhof *et al.*, 2009; Sandin *et al.*, 2004). However, pet avoidance is believed to be a confounding factor in studies on associations of exposure to pets and development of allergic disease (Eller *et al.*, 2008), and suggested to account for the putative 'protective' effect of pet keeping observed in some studies (Almqvist *et al.*, 2003; Brunekreef *et al.*, 1992). In addition to removing the allergen source, environmental control practices can also be performed to reduce the allergen exposure. There are several common recommendations for allergen avoidance, such as vacuuming, washing the bed linens and clothing regularly, and the use of air filters and dehumidifiers (Tovey, 2008). However, these recommendations for reducing the allergen exposure may differ in their efficiency in regard to different sources of indoor allergens (Tovey, 2008).

Allergic sensitization to cat and dog allergens are commonly observed in individuals who do not have pets at home (Chen *et al.*, 2007; Perzanowski *et al.*, 1999). In one study it was found that the prevalence of sensitization to pets was linked to the abundance of pets in the community (Braback *et al.*, 2001). Since these allergens are easily bound to textiles (D'Amato *et al.*, 1997) and human hair (Karlsson and Renstrom, 2005), pet allergen exposure in public areas may be dependent on the community prevalence of furry pets (Almqvist *et al.*, 1999; Instanes *et al.*, 2005; Perzanowski *et al.*, 1999). Children without pets at home may thus be exposed to higher allergen levels at kindergarten and school than at home (Almqvist *et al.*, 1999; Perzanowski *et al.*, 1999).

2.3.6 Endotoxins and ß(1,3)-glucans

Many microorganisms inherently possess adjuvant activity in the form of immunostimulatory molecules such as lipopolysaccharide (endotoxin) and muramyl dipeptide (Delves and Roitt, 2000). Viable and nonviable parts of microorganisms are found in varying concentrations in many indoor and outdoor environments. Microbial exposures early in life are hypothesized to prevent allergic sensitization and asthma by promoting a Th1-type (non-allergic) immune development (Abraham *et al.*, 2005; Holt and Jones, 2000). However, exposure to microbial components may also promote a Th2-type (allergenic) immune development. One possibility

is a gene-environment effect, as previously discussed (Bruce *et al.*, 2009; Ege *et al.*, 2007). Environmental exposure to microbial products may, therefore, have a crucial role in the development of a child's immune response.

Endotoxin is a component of the cell wall of all gram-negative bacteria, and is therefore ubiquitous in the environment. The paradox of endotoxin is that higher levels of exposure early in life may inhibit the development of allergy and asthma (Abraham *et al.*, 2005; Celedon *et al.*, 2007; Douwes *et al.*, 2006; Schram *et al.*, 2005; von Mutius *et al.*, 2000), but exposure to high levels of endotoxin is also a frequent cause of asthma-like symptoms in a wide range of occupational settings such as cotton and textile processing, and livestock farming (Bakirci *et al.*, 2007; Thorne *et al.*, 2004). Asthmatics are particularly sensitive to inhaled endotoxin, and inhalation induces both immediate and sustained airflow obstruction (Michel *et al.*, 1997; Rylander *et al.*, 1989). These findings are in line with the reports on increased risk of wheezing in high-risk children exposed to endotoxin (Gillespie *et al.*, 2006; Park *et al.*, 2001; Perzanowski *et al.*, 2006).

It is likely that other microbial agents, like mould components, may affect immune responses (Douwes *et al.*, 2006). ß(1,3)-glucans, a cell wall constituents of most fungi, have strong immunomodulary effects (Novak and Vetvicka, 2008). ß(1,3)-glucans have been found to increase the IgE response to co-administrated allergen (adjuvant effect) (Instanes *et al.*, 2004; Ormstad *et al.*, 2000), but the susceptibility may differ between high- and low-risk children (Belanger *et al.*, 2003). Nevertheless, the evidence of a causal role for ß(1,3)-glucan in allergic disease development is still very limited (Douwes, 2005).

ß(1,3)-glucans have also been suggested as an indicator of moulds in the indoor environment, making it difficult to determine whether it is ß(1,3)-glucans itself, the mould exposure or other concomitant exposures such as from wet materials that are actually responsible for the observed effects in epidemiological studies (Rylander *et al.*, 1998). ß(1,3)-glucans can also derive from some bacteria and several higher and lower plants, and are therefore an uncertain bio-marker of mould exposure (Douwes *et al.*, 1999). Similar problems may occur as the presence of pets, and also vermins such as mice and cockroaches (Bischof *et al.*, 2002; Heinrich *et al.*, 2001) are suggested to be important determinants of endotoxin (Bischof *et al.*, 2002; Gehring *et al.*, 2004; Giovannangelo *et al.*, 2007; Heinrich *et al.*, 2001) and ß(1,3)-glucans levels in house dust (Gehring *et al.*, 2001). In addition, both higher ß(1,3)-glucan and endotoxin levels are associated with floor carpeting, the number of individuals in the home, and lower frequency of vacuum and dust cleaning (Bischof *et al.*, 2002; Gehring *et*

al., 2001). However, these are factors that are also likely to be associated with elevated levels of cat, dog and HDM allergens.

2.4 Epidemiological methods

2.4.1 Study designs

The objectives of epidemiological studies are to monitor the extent and the natural history and prognosis of a disease, and identify causes and risk factors that may lead to new preventive and therapeutic strategies (Gordis, 2000). Epidemiology is an extensive field and the following discussion will be focused on the use of epidemiology to identify factors implicated in disease onset.

The most commonly used study designs to identify associations between exposure and disease in environmental epidemiology are cohort and case-control studies. In prospective cohort studies the population is identified at the start of the study. Exposure and non-exposure are ascertained as they occur during the study until the point when disease develops or not (Gordis, 2000). Depending on the outcome of interest, it may take many years to conduct such a study. An alternative approach is to perform a retrospective cohort study (a historical cohort). The exposure is ascertained from past records and outcome (disease) is recorded at the beginning of the study. However, the term retrospective has often been incorrectly used for case-control studies, as the term suggests that calendar time is the characteristic that distinguishes case-control from cohort designs. What distinguishes the two study designs is whether the study begins with diseased and non-diseased people (case-control study) or with exposed and non-exposed people (cohort study). A nested case-control study is a hybrid design in which a case-control study is established within a cohort study.

In cross-sectional studies both exposure and disease outcome are determined simultaneously. But a cross-sectional study may also be retrospective in regard to the exposure.

Cohort studies are usually preferred when the goal is to detect associations between exposure and disease development, but differences in design such as population-based, high-risk (children with atopic parents) and enriched cohorts (a higher prevalence of high-risk children than in a normal population) and different follow-up time between exposure and disease may lead to heterogeneity in the observed associations.

Although epidemiological studies have the advantage of real-life exposure situations and may accumulate information on exposure-response relationships, experimental (animal) models and in vitro studies are usually necessary to prove cause-effect relationship. However,

epidemiological studies can find associations between exposure and disease, but do not prove any cause-effect relationships. Intervention studies may support causal relationships between exposure and disease, because there is a certain control of the exposure. Moreover, epidemiological studies showing dose-response relationships may also give convincing evidence of causal relationship, such as between male lung cancer and the number of daily cigarettes smoked during the 10 years preceding the onset of the illness (Doll and Hill, 1952). Furthermore, accumulated information on exposure-response associations provided by epidemiological studies may in some cases lead to the identification of cause-effect relationships.

2.4.2 Issues of concern

Important issues leading to the lack of ascertainment of causality in epidemiological studies are the problems of confounding, bias and interactions and stating temporality (Gordis, 2000).

A confounder is an additional variable which may mask or distort the apparent effect of the exposure on the disease (Gordis, 2000). Typical confounders are age and gender. However, depending on the research question, gender could also be an important explanatory factor on its own.

Interaction is a real difference in the effect of exposure in various subgroups, in which the effect of one variable depends on the value of another (Gordis, 2000). A typical example is smoking and asbestos which both lead to lung cancer, but where the effect of both exposures simultaneously is multiplicative and not additive to the risk of lung-cancer. Confounders and interactions are usually dealt with by statistical methods, typically by applying multivariate models to control for perceived or established confounders and interactions. If an interaction is confirmed, the data can also be stratified by the variable responsible for the interaction effect.

Bias is any systematic error in the design, conduct or analysis of a study that results in an incorrect estimate of the association between the exposure and the disease (Gordis, 2000). Well-known examples of bias are selection bias, which is an important problem in case-control studies, such as inclusion of hospital cases that may represent the more severe cases of disease. Participation bias may occur in cohort studies if the individuals who do not participate differ from those who do, in regard to socio-economic, cultural, life-style and medical characteristics. Selection and participation bias affect the possibility to generalise the finding because the participants do not represent the population of interest. Information and in particular recall bias are of major concern in retrospective study designs. Limitations in recall

may occur if the interviewed subjects do not recall or simply do not have the information being requested. In addition, recall bias occur when individuals with a disease remember their history of either or both disease and exposure better than individuals without disease. This was demonstrated in the German MAS study, where the parents showed improved recall of their own asthma and hay-fever symptoms after their child had developed allergic symptoms (Kulig *et al.*, 2000). However, if all subjects are affected randomly, no bias is introduced.

The temporality condition requires that the exposure has to precede the outcome, which is an undisputable factor when trying to establish causality. If the researcher has to go back in time to collect information on exposure and onset of disease (retrospective studies), the temporal link between exposure and disease may be unclear. In prospective cohort studies the temporality criterion is usually fulfilled as the observation period starts with exposed and un-exposed individuals before the disease (outcome) occurs.

Nevertheless, epidemiology has the advantage of a real-life exposure situation. This is important since the effect of a complex mixture of environmental agents may be different than the sum of the effects of the individual components. In addition, the exposure concentrations reflect the actual range of human exposure.

In view of the unanswered questions related to onset of allergic disease in childhood, the prospective birth cohort the Environment and Childhood Asthma Study in Oslo has the propensity to answer some of the environmental, genetic and life-style factors implicated in disease development.

3 Aims of the study

The overall objective was to obtain more knowledge about the association between exposure to allergens and microbial factors in the indoor environment and the development of allergic diseases in childhood. In this context, the term ‘allergic diseases’, refers to allergy-related diseases with and without demonstrated allergic sensitization.

The specific questions we sought to answer were:

1. Is pet exposure influenced by a family history of allergic disease?
2. Is exposure to furred pets associated with development of allergic diseases?
3. Does exposure to house dust mite and microbial components influence the development of allergic diseases?
4. Do boys and girls differ with respect to exposure and in exposure-disease associations?

4 Subjects and methods

4.1 Study design

The present thesis is based on results from the prospective birth cohort *the Environment and Childhood Asthma Study.* The ECA study was established in 1992 as a collaborative study between Ullevål Hospital and the Norwegian Institute of Public Health. One of the main aims of the ECA study was to investigate associations between possible environmental risk factors and the development of childhood asthma. At the maternity wards of the two municipal hospitals in Oslo (Ullevål Hospital and Aker Hospital) 3754 infants (75% of all eligible) (Nafstad *et al.*, 1996) were recruited at birth during 15 months from January 1st 1992.
The infants were recruited on the following the criteria:

Inclusion criteria:

- Birth weight more than 2000 g.
- Absence of any illness likely to impair respiration (severe respiratory, cardiovascular, neuromuscular or metabolic disease)
- No requirement for assisted ventilation or oxygen therapy beyond 6 hours after birth

Exclusion criteria:

- Plans to move out of Oslo the next six months
- Insufficient language comprehension by the parents to be able to complete questionnaires

Lung function measurements (tidal flow-volume loops and passive respiratory mechanics) were performed shortly after birth (mean age (±SD): 2.7 (0.9) days) in 802 infants at the largest maternity ward at Ullevål Hospital (Lodrup Carlsen *et al.*, 1994). The parents completed extensive questionnaires at birth and thereafter every sixth months for the first 2 years of life. At 2 years of age, all the participating families were invited to submit dust samples from their home.

A nested case-control study was established with cases identified by the questionnaires and clinical registration cards with doctors documenting signs of bronchial obstruction (BO) as well as direct referrals from well-baby clinics, hospital admissions and family doctors. A case was a child with recurrent BO (rBO) defined by at least two episodes of BO or BO lasting more than four months before 2 years of age. At least one episode of BO had to be confirmed by a doctor. The controls were children without BO born closest in time to the

case. The children (n=562) attended study follow-up investigations 1-2 times by 2 years of age.

At 10 years, all the children who had attended at least one of the clinical visits the first 2 years of life and/or had lung function measurements at birth were invited to a follow-up study (figure 3). A total of 1019/1215 (84%) of the eligible children attended the two days of examination (within a week) including lung function assessment, testing of bronchial hyperresponsiveness, exercise test by treadmill running, skin prick tests, collection of blood and urine samples, collection of parent-collected dust from the child's mattress and an extensive interview of the parents performed by the study doctors.

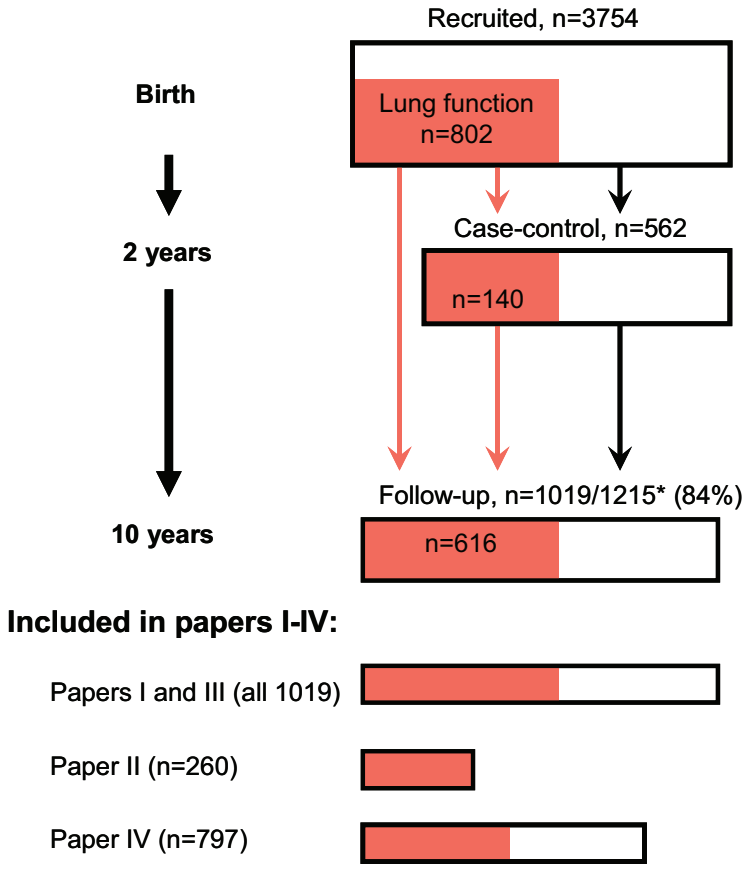

***Figure 3**: Simplified study design of the ECA study, and number of participants included in Papers I-IV. *Invited children were all traceable children with either lung function measured at birth (n=802) and/or included in the case-control group at 2 years (n=562), in total 1215.*

4.2 Subjects

Papers I and III

The study population of Papers I and III include all 1019 children (551 boys, 468 girls) who attended the 10-year follow-up study (mean age (± SD): 10.8 (0.83) years). The 1019 participating children were at birth comparable to the 2735 non-included children with regard to gender, parental education, type of housing and parental smoking and pet keeping. However, due to the nested case-control study, the population was enriched with allergic disease, with significantly more parents or siblings having rhinoconjunctivitis (31.7%, vs. 26.9% p = 0.004) and asthma (17.7% vs. 14.8%, p = 0.031).

Paper II

The study population of Paper II includes 260 (131 boys, 129 girls) of the 616 children attending the 10-year follow-up, who had lung function measured at birth *and* an available dust sample from 2 years of age. Except higher parental education level, they were similar to the 365 children without dust samples in regard to birth characteristics and pet keeping during the first 2 years of life. At 10 years of age, the 260 included children were less often sensitized to cat, but were otherwise comparable to the non-included children (10.4% and 16.9%, respectively, P=0.02).

Paper IV

Paper IV includes the 797 of the 1019 children (437 boys, 369 girls) with an available dust sample from 10 years of age. At 10 years of age included children had less cats at home (18% vs 26%, p=0.007), less parents who smoked (38% vs 47%, p=0.02) and parents with a higher education than the 222 non-included children (without dust samples). They were otherwise comparable in regard to the children's and the parents' allergic disease, family income, type of housing, other pets than cats, and reported pet avoidance.

4.3 Methods

4.3.1 Questionnaires and clinical examinations

Parental questionnaire and interview

The semi-annual questionnaires during the first 2 years of life included questions about symptoms of respiratory and other allergic diseases in the index child, allergic diseases in parents and siblings, socio-economic status (parental education, income and housing etc.) and indoor environmental exposures like pet keeping, dampness, and parental smoking.

At the 10 year follow-up the parents responded to a structured interview completed by the study doctor, including core ISAAC questions about symptoms of lower and upper respiratory diseases, having a doctor's diagnosis of asthma, reported doctor confirmed allergic sensitization, allergic diseases in parents and siblings, socioeconomic factors, pet keeping and pet avoidance, as well as other detailed questions regarding home environmental factors.

Lung function measurements

Lung function measurements were performed at a mean age of 2.7 days after birth with tidal flow volume loops and passive respiratory mechanics by the SensorMedics 2600 system (Yorba Linda, Anaheim, USA), as described in detail elsewhere (Lodrup Carlsen *et al.*, 1994).

At 10 years of age lung function was measured by forced expiratory flow volume loops according to European standards (Quanjer *et al.*, 1993) on a Sensor Medics Vmax 20c instrument (SensorMedics Diagnostics, Yorba Linda, CA, USA) on two occasions within a week. The reported values were the best values obtained of these two efforts and included forced expiratory volume in one second (FEV_1) and forced expiratory flow at 50% of forced vital capacity (FEF_{50}) as percent predicted [reference values of healthy 10-year old children from the ECA-study obtained by methods described by Stanojevic et al. (Stanojevic *et al.*, 2009)].

Assessment of bronchial hyperresponsiveness

At 10 years of age airway bronchial hyperresponsiveness (BHR) was assessed by both a direct method (methacholine challenge test) and indirect method (exercise challenge). Both tests were performed on separate days within the same week. The methacholine challenge test was performed according to international guidelines (Crapo *et al.*, 2000) by inhaling doubling doses of methacholine nebulized by the inhalation triggered Spira Dosimeter (Spira Respiratory Care Center Ltd, Hämeenlinna, Finland) until a fall in FEV_1 of 20% from the (post-saline) baseline value or until the maximum dose of methacholine (22.4 µmol) was reached. The exercise challenge test was performed by a 6-8 minutes treadmill run according to a standardized protocol (Carlsen *et al.*, 2000) and lung function was measured before and 3, 6, 10, 15 and 20 minutes after the test.

Skin prick tests and specific IgE at 10 years of age

Skin prick testing were performed according to the European standards (Dreborg and Frew, 1993) with the following standardized allergen extracts from ALK (Soluprick®, ALK-Abello,

Denmark): Domestic mites (*Dematophagoides pteronyssinus* and *D. farinae*), German cockroach, dog, cat and rabbit allergens, birch, timothy (grass) and mugwort pollen, moulds (*Cladosporium* and *Alternaria*), egg white, milk, peanut and codfish. SPT were considered positive with a wheal diameter at least 3 mm larger than the negative control (saline).

Total and specific serum IgE (IgE and sIgE, respectively) were analysed with a radioallergosorbent fluorescence immunoassay (ImmunoCAP® system, Phadia, Uppsala, Sweden). Specific IgE was analysed with the same panel of allergens as for SPT as well as the panel test "Phadiatop", and considered increased with sIgE ≥0.35 kU/L.

4.3.2 Collection and extraction of dust samples

Paper II

The dust samples were collected by the parents according to detailed written instructions. They were asked to use new vacuum cleaner bags, vacuum the floor of the living area of the house and collect the entire sample of dust from the bags. The dust samples were shipped in plastic bags to the Norwegian Institute of Public Health and stored at -20°C until extraction and analysis. Endotoxin, allergens and ß(1,3)-glucans were extracted sequentially from un-sieved dust by methods adopted from the Institute for Risk Assessment Science at University of Utrecht, the Netherlands (Schram *et al.*, 2005). In short, endotoxin was extracted in pyrogen-free water with 0.05% Tween-20. The volume varied according to the net weight of the dust. After removal of supernatant for endotoxin analyses, the remaining dust pellet was re-suspended into phosphate-buffered saline with 0.05% Tween-20 (PBT) for allergen extraction. Finally, the pellet was re-suspended and autoclaved at 120°C for ß(1,3)-glucans extraction. The samples were stored at -20ºC until analysis.

Paper IV

Dust from the child's bed mattress was collected by the parents with vacuum cleaners equipped with a special dust collector (P-B Miljø A/S, Bjerringbro, Denmark) (Dreborg *et al.*, 1995). The families were urged not to change bed linen for one week prior to the sampling of the mattress dust. The families were instructed to vacuum the mattress for four minutes, and to report the size of the mattress and the effect of the vacuum cleaner. At the laboratory, the dust was vacuumed (sieved) through a filter with pore size 0.3 µm, and the fine fraction of the dust was weighed and extracted in phosphate-buffered saline with 0.02% NaN_3 and agitation at room temperature overnight. The supernatant was the stored at -20ºC until analysis.

4.3.3 Analyses of allergens, endotoxin and ß(1,3)-glucans in dust samples

In Papers II and IV the concentration of cat (*Felis domesticus,* Fel d1), dog (*Canis familiaris,* Can f1) and house dust mite (*Dermatophagoides pteronyssinus,* Der p1) allergens were determined by enzyme-linked immunosorbent assay (ELISA) following the manufacturer's instructions (Indoor Biotechnologies, Clewyd, UK) (Chapman *et al.*, 1987). Concentrations were expressed as µg allergen per gram dust.

Endotoxin (Paper II) was analyzed with the kinetic chromogenic Limulus Amebocyte Lysate (LAL) test, using the same batch of LAL reagents and standards for all analyses (BioWhittaker, Walkersville, USA, LAL lysate lot no. FL119G, Lipopolysaccharides (LPS) standard lot no. FL0835). Thirty percent of the samples were tested in duplicate, with a mean coefficient of variation of 4.8%. Concentration was expressed as endotoxin units (EU) per milligram dust.

ß(1,3)-glucans (Paper II) were measured with an inhibition enzyme immuno assay (EIA) (Douwes *et al.*, 1996) with affinity-purified anti-ß(1,3)-glucan antibodies (Batch 9819, provided by Dr. G. Doekes, IRAS, Utrecht, The Netherlands). The concentration was expressed as µg ß(1,3)-glucan per gram dust.

The extractions and analysing procedures are described more thoroughly in the individual papers.

4.3.4 Definitions and outcomes at 10 years

BHR was defined as PD_{20} methacholine ≤8 µmol in Paper II and separated into BHR (PD_{20} ≤8 µmol) and *severe BHR* (PD_{20} ≤1 µmol) in Paper III. BHR was reported as a continuous variable with the methacholine *dose-response slope (DRS)* calculated from each individual (per cent decline in FEV_1 after the final dose of methacholine divided by the cumulative dose of methacholine inhaled (O'Connor *et al.*, 1987)) in Papers III and IV.

The exercise test (Papers III and for defining current asthma) was considered positive for *exercise-induced bronchoconstriction* (*EIB*) with a fall in FEV_1≥10% of baseline FEV_1 measured 3-20 minutes after running ceased.

A history of asthma (Paper II) was defined in children by the presence of at least two of the three criteria; asthma symptoms, doctor's diagnosis of asthma, and use of asthma medication (Lodrup Carlsen *et al.*, 2006a).

Current asthma (Papers II - IV) at 10 years of age was established when a child with a history of asthma had either had symptoms or used asthma medication within the last 12

months and/or had a positive exercise challenge test at the 10 year follow-up visit (Lodrup Carlsen *et al.*, 2006a).

Allergic sensitization (Paper II) was defined by at least one positive SPT and/or sIgE to any of the allergens tested.

Current rhinitis (Papers III and IV) was present in a child reporting at least one of the following symptoms (without a cold) during the last 12 months: runny nose, blocked nose, or sneezing. C*onjuntivitis* required a report of itchy/runny eyes during the last 12 months. Other outcomes related to rhinitis (Papers III and IV) were retrieved from the parental interview at 10 years of age, and are presented in table 1.

Current rhinitis with allergic sensitization (Papers III and IV) was defined in a child with current rhinitis combined with at least one positive SPT and/or sIgE to any of the *inhalant* allergens tested: *seasonal* (pollens or moulds) or *perennial* (house dust mites, cat, dog, rabbit or cockroach). *Mixed sensitization* was defined with SPT/IgE positive to at least one seasonal *and* one perennial allergen.

Current wheeze (Papers III and IV), and *otitis media* and *atopic eczema* (Paper III) were defined by the presence of these outcomes (as reported by the parent in the interview) during the last 12 months.

Variables regarding pet keeping (Papers I, II and IV), pet avoidance, socioeconomic factors, and atopic disease were retrieved from questionnaires and interview and are presented in table 2.

Table 1 *Questions related to symptoms, triggers and medical treatment of rhinitis from the structured parental interview at the 10-year follow-up [the questions are translated from Norwegian (Appendix B)]*

INTERVIEW QUESTIONS	**OUTCOMES**
Has the child had any of the following symptoms during the last 12 months (without a cold): a) runny nose, b) blocked nose, c) sneezing, d) itchy/runny eyes	Rhinitis (a, b and/or c) Conjunctivitis (d)
What causes the eye/nose symptoms? a) not known b) dog c) oat d) other furry pets e) other causes f) pollen from trees g) other pollens h) temperature changes/physical activity	Unknown cause of symptoms (a) Symptoms related to furry pets (b,c, d) Symptoms related to pollens (f, g) Symptoms related to furry pets *and* pollens (b, c d *and* b, c) Symptoms related to other causes than allergens (e, h)
Does the child have symptoms of rhinitis during a) the whole year, b) spring/summer or c) autumn/winter	Time of year when symptoms occur
How much impact have these symptoms had on daily life activities? a) none b) some c) moderate d) severe	Impact on daily activities
Has the child ever been given the diagnosis hayfever/allergic rhinitis?	Doctor diagnosed hayfever ever
What kind of treatment has the child received due to symptoms of rhinitis? a) none b) don't know c) topical antihistamines d) systemic antihistamines e) topical cortocisteroids f) systemic cortocisteroids g) sodium cromoglycate h) leukotriene antagonist i) immunotherapy j) homeopathy k) other alternative medicine	Received medical treatment due to rhinitis (b-i) Alternative medicine (j-k)

Table 2 *Questions concerning allergic disease, socio-economic factors and pet-keeping from questionnaires at birth till 2 years of age and from parental interview at the 10-year follow-up [translated from Norwegian (Appendix A and B)]*

AGE	QUESTIONS	OUTCOME
Allergic disease in the index child and child's family		
6, 12, 18 and 24 months	Has the child had atopic eczema during the last 6 months?	Atopic eczema by age 2
10 years	Has the child ever had any of the following symptoms irrespective of a cold: runny nose, blocked nose, sneezing and/or itching/runny eyes?	Allergic rhinoconjunctivitis ever
	Has the child ever had any allergies confirmed by a doctor? (not including food-allergies)	
	Has the child ever had a diagnosis of asthma confirmed by a doctor?	Ever diagnosed with asthma
	Has the child previously experienced respiratory (wheezing) symptoms caused by furry pets?	Wheezing symptoms due to furry pets
Birth	Have any of the following family members ever had asthma: Father, mother or siblings?	Siblings or parents with a history of asthma
	Have any of the following family members ever had rhinoconjunctivitis: Father, mother or siblings?	Siblings or parents with a history of rhinoconjunctivitis
Environmental and socio-economic factors		
Birth	What was the total family gross income last year? 1:<100, 2:100-199, 3:200-299, 4:300-500,5:>500 in 1000 NOK	Family income
	Do mother and/or father smoke at home (sometimes or daily)?	Parental smoking at birth
10 years	Did mother and/or father smoke at home a) before school-age (age 6-7) b) after school-age	Parental smoking (after child's birth)
Birth and 10 years	Which type of house does the child live in? a) flat b) detached/semi-detached house.	Housing
Birth	Father and mother highest completed education: a) compulsory (± 1-2 year county college) b) college c) university	Parents' education (highest education by either parent)
10 years	With whom does the child live with more than >50% of the time? a) mother and father b) mother c) father	Single parenthood
	Has the family avoided pets or removed pets due to allergy in the family and/or to prevent the child from developing allergies?	Reported pet avoidance
Birth, 6,12,18,24 months and 10 years	Do you keep furry pets at home? If yes: a) cat b) dog c) other types of furry pets (rodents)	Cats, dogs or other furry pets at birth Change in pet ownership from birth to age 10
10 years	Did you keep cats, dogs or other furry pets at home during the child's first year of life?	Retrospective information on pet keeping during first year of life

4.4 Statistical methods

Categorical variables were compared by Pearson's chi-square test or Fisher's exact test when small cell numbers. Differences in continuous variables between multiple groups were assessed by one-way ANOVA (Paper IV), or by the nonparametric Mann-Whitney U test, and correlations were given by Spearman's rho (ρ) coefficient (Paper II). Log-transformed categorical variables were presented as geometric mean (GM) and 95% confidence intervals (95% CI).

The procedure used for the multivariate logistic regression models in Papers I and II was backward elimination technique, starting with all the candidate variables in the model (Hosmer D.W. and Lemeshow S, 2000). All the predictor variables had equal entry status in the models. At each step, the variable that was the least significant was manually removed. This process continued until only statistically significant variables remained in the model. The condition of linearity of the continuous variables in Papers II and IV was tested with a fractional polynomial analysis (Sauerbrei and Royston, 2009) which supported treating the cat and dog allergens, endotoxin and ß(1,3)-glucans variables as continuous and linear in the models. For the linear regression models in Paper II, the condition of normally distributed residuals of the dependent variable was fulfilled. In Paper IV, a linear regression with DRS as outcome variable was performed. DRS was log-transformed due to the highly skewed distribution of the variable, and due to the violation of normality and homoscedasticity assumptions, a robust regression was performed, using Huber's method (Hamilton, 1991). The values resulting from the linear regression model were back-transformed and the results presented per increase in DRS. Furthermore, all multivariate models were tested for confounding and interactions.

The limit of statistical significance was set to 0.05 (5%). Statistical analyses were performed with Statistical Package for Social Sciences (SPSS version 14.0, SPSS Inc., Chicago, Illinois, USA) and Statistical Analysis System (SAS version 9.1.3, SAS Institute Cary, NC, USA) and Number Cruncher Statistical System (NCSS version 2007, Kaysville, Utah).

Categorization of lung function (Paper II)

t_{PTEF}/t_E and V_E (expiratory volume) from the lung function measurements at birth were dichotomized according to the median value of 0.29 and 24.2, respectively. For calculation of *change in lung function* from birth (t_{PTEF}/t_E) to age 10 (FEV_1 or FEF_{50}), z-scores based upon mean and SD of the healthy subjects were calculated. The z-scores describe each child's

position relative to the healthy individuals in the population. The reference value for the z-scores at birth were calculated for all children (as they were healthy at the time) and at 10 years for all children without any lower respiratory tract infections during the first 2 years of life and without a history of asthma (Haland *et al.*, 2009).

Prevalence of rhinitis (Paper III)

To adjust for the enrichment of children with bronchial obstruction during the first 2 years of life, an overall prevalence of rhinitis was calculated by the weighted mean of the prevalence of rhinitis for the following groups: i) rBO by age two, ii) controls, and iii) the children with lung-function measured at birth who were not included in the case-control group. The rhinitis prevalence for these three groups was calculated for girls and boys separately and the weight was the number of children in each group. The prevalence was reported with confidence intervals.

4.5 Ethical issues

Written informed consent was obtained from parents of all the included infants at the maternity ward before inclusion in the ECA study. Renewal of the consent was obtained at enrolment into the 10 year follow-up study after written information to the parents as well as the child. The study was approved by the Norwegian Data Inspectorate and the Committee for Medical and Health Research made no objections to the study. The study is registered in the Norwegian Biobank Registry.

5 Results

5.1 Pet exposure and family history of allergic disease

At birth, 7.9% and 9.3% of the 1019 families had cats and dogs, respectively. Pet ownership was more common at 10 years of age at which time, 19.8% of the families reported having cats and 15.2% having dogs. The biggest change was seen for furry pets (rodents) other than cats and dogs from 1.3% at birth up to 17.9% at the age of 10. Overall, 319 families reported pet avoidance, of which 70% had never kept pets, 8% had given up pets, and 22% had a least one furry pet at the time of the follow-up (Paper I). Of the 215 families keeping furry pets during the child's first year of life according to questionnaires at birth, 6 and 12 months, 24% failed to report these pets at the 10-year follow-up.

The parents' and siblings' history of asthma and rhinoconjunctivitis did not show a statistically significant association with pet keeping at birth, with pet removal or with pet acquisition at 10 years of age. Nor was pet removal or pet acquisition related to early symptoms of rBO or a doctor's diagnosis of asthma in the index child (Paper I). However, acquisition of cats and dogs after birth was less likely if the index child had allergic rhinoconjunctivitis ever confirmed by a doctor. Also at 10 years of age, children with current rhinitis and/or allergic sensitization had significantly lower cat and dog allergen levels in their mattress dust compared to children without current rhinitis and allergic sensitization (Paper IV). In the multivariate analysis, reported pet avoidance, but not cat and dog allergen levels in mattress dust, was significantly associated with current rhinitis. The majority of children with allergic sensitization at the 10-year follow-up study were already aware of their sensitization status, as 90% reported a previously confirmed doctor's diagnosis of allergy to inhalant allergens (Paper IV).

Socioeconomic factors like single parenthood, smoking and education level were also strong determinants of pet keeping (Paper I). Parental smoking was associated with cat keeping at the child's birth as well as with acquisition of cats and/or dogs later in childhood. Children living with a single parent (n=246) by the age of 10 years (78% single mothers) were more likely to have acquired cats, but also to have removed their dog. On the other hand, high parental education was associated with both reported pet avoidance and with not having dogs at the time of the child's birth.

5.2 Pet exposure and allergic disease

Overall, 34.4% of the children were sensitized to at least one inhalant allergen at 10 years of age, and 17.8% were sensitized to both seasonal and perennial allergens (Paper III). Timothy grass, birch, dog, cat and mugwort were the most prevalent sensitizing inhalant allergens (Figure 4). Twenty-five per cent of the 1019 children attending the 10-year follow-up had current rhinitis (n=254), and 72.8% of these were sensitized to at least one allergen.

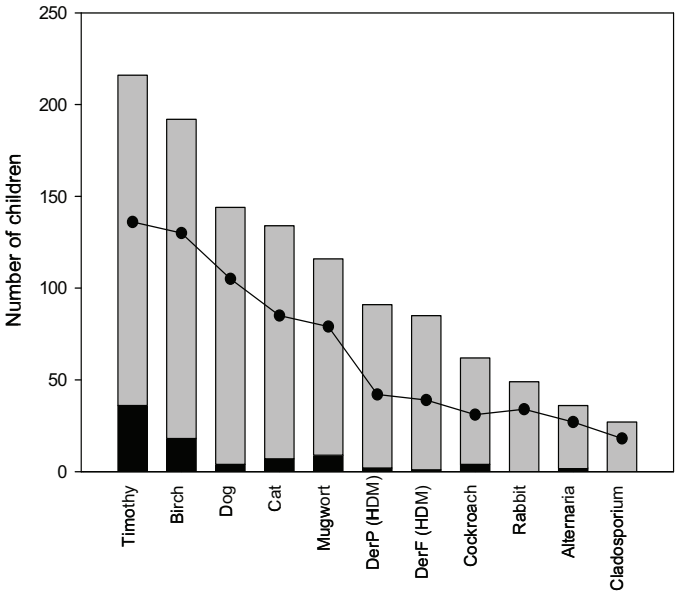

Figure 4: *Grey bars represents the number of children with sensitization to each of the specific allergens in increasing order from the most common (timothy grass) to the least common (Cladosporium), and the black fields of the bars represents the number of children with mono-sensitization. The dots represent the number of children for each specific sensitizing allergen who also have current rhinitis.*

The majority of children (193/254) reported allergen-triggered rhinitis, of whom 15 children reported symptoms triggered by furry pets only, 53 by pollen and furry pets, and 125 by pollen only. Children with symptoms related both to pollen and furry pets more frequently had co-morbidities of asthma and wheeze and more severe BHR ($PD_{20} \leq 1$) than children with pollen-triggered rhinitis only (all $p \leq 0.01$). However, among the children who had become sensitized at the 10-year follow-up, 50.4% of those who reported pollens as the only trigger of rhinitis, were in fact sensitized also to non-seasonal allergens (mostly to cat (37%) and/or dog

(24%)), whereas 28.8% were sensitized to seasonal allergens alone. Notably, 18.4% of the children with reported pollen-triggered rhinitis had no allergic sensitization (Paper III). The children who were unable to relate their symptoms to any specific triggers had significantly higher dog allergen concentrations in their mattress dust (GM: 0.67 (95% CI: 0.37, 1.22) µg/g dust) compared to the children with recognised perceived rhinitis triggers (0.34 (0.25, 0.46)), p=0.050 (adjusted for dog ownership) (Paper IV). However, assessing influence upon current rhinitis symptoms, impact the symptoms had on daily life activities, and perceived triggers of rhinitis, the multivariate analyses adjusted for gender, allergic sensitization, current asthma and reported pet avoidance, demonstrated no significant effect of the level of cat and dog allergens.

Assessing the risk of allergic disease at 10 years of age by pet allergen exposure in early childhood demonstrated that only cat allergen levels were associated with later disease. Thus, the risk of current asthma at 10 years of age increased with an odds ratio of 1.20 (95% CI: 1.01, 1.43), p=0.04, per 10 µg increase in cat allergen levels at 2 years of age (Paper II). However, stratifying by allergic sensitization, the association was significant only for children *without* allergic sensitization (adjusted OR: 1.32 (1.07, 1.62), p=0.009). Dog allergen levels at 2 years of age, however, did not modify the risk of allergic disease at 10 years of age. Moreover, the children who were sensitized to cat and dog allergen at 10 years of age were most likely to never having kept cats (85%) and dogs (91%) at home (Paper II).

5.3 The influence of house dust mite and microbial components on the development of allergic disease

The levels of endotoxin and ß(1,3)-glucan at the age of 2 did not modify the risk of allergic sensitization, asthma, or BHR at 10 years of age (Paper II). Although FEV_1 % predicted at 10 years of age decreased with increasing levels of endotoxin in the home at 2 years of age, the effect was small and disappeared after removing one extreme outlier from the analyses. The levels of endotoxin and ß(1,3)-glucan measured in house dust at 2 years of age was not associated with pet keeping, and the correlations between cat and dog allergens, ß(1,3)-glucan and endotoxin were weak for all groups (all $\rho \leq 0.17$).

Detection of HDM allergen, Der p1, was uncommon in the present study. Only 1.5% of the house dust samples from 2 years of age (Paper II) and 12.3% of the mattress dust samples from 10 years of age (Paper IV) had detectable levels of Der p1. Nevertheless, the 8.8% of the children who were sensitized to Der p1 at the age of 10, had significantly higher Der p1 levels

in their mattress dust compared to those without Der p1 sensitization, (GM: 0.21 µg/g dust (95% CI: 0.16, 0.28) and (0.16 µg/g dust (0.15, 0.17)), p=0.004, respectively (Paper IV).

5.4 Gender effects on exposure and exposure-disease association

At 10 years of age boys were significantly more likely to have current asthma (p=0.002), current rhinitis (p=0.001) and to be sensitized to inhalant allergens (p<0.001) as compared to girls, whereas 10.1% of the girls and 7.4% of the boys had symptoms of rhinitis without detected allergic sensitization (p=0.3) (Paper IV).

The predicted probability of asthma at 10 years of age related to cat allergen levels at home at 2 years of age (Paper II) was higher for boys than for girls (Figure 5).

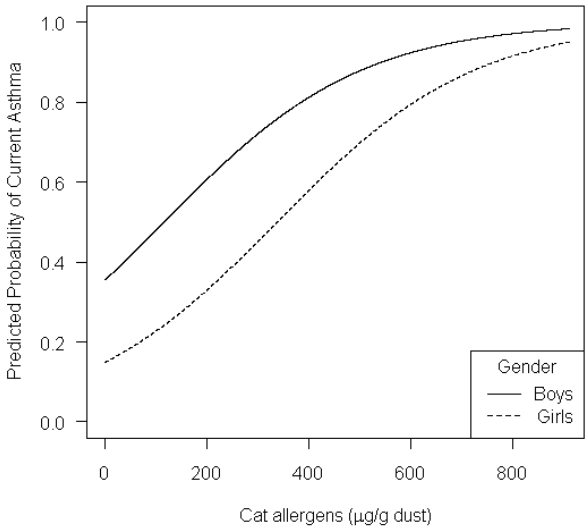

Figure 5: *The predicted probability of current asthma at age 10 for boys and girls based on risk calculation (OR) in relation to increase in cat allergen exposure at 2 years of age, adjusted for alcohol in pregnancy (reference:no), parental rhinoconjunctivitis at birth (reference:no) and parental education (reference category 4: college, vocational).*

Families in which the index child was a girl were more likely to acquire other furry pets than cats or dogs, compared to families with boys (adjusted OR: 1.85, 95% CI: 1.33, 2.56) (Paper I). At 10 years of age, girls tended to report more cats at home and regular contact with cats (p=0.03), but no significant gender difference was seen for dog contact or dog ownership

(p=0.4) (Paper IV). However, regardless of pet ownership, girls had higher levels of cat and dog allergens in their mattresses compared to boys (both p≤ 0.001) (Paper IV).

The association between cat and dog allergen levels in mattress dust and current rhinitis symptoms did not differ significantly between boys and girls, whereas HDM allergen levels interacted significantly with gender in relation to current rhinitis (Paper IV). The latter association was seen for girls only, where adjusted odds ratio of current rhinitis symptoms increased with 1.20 (95% CI: 1.01, 1.42) per 1 μg/g increase in HDM allergen levels (p=0.037). No significant association was seen for boys (p=0.9).

6 Methodological considerations

6.1 Dust sampling

Comparing allergen concentrations and microbial components measured in dust samples from different studies is challenging due to variation in sampling, storage and extraction procedures. As an example, HDM allergen is most common in mattresses, whereas the living room and kitchen may be more appropriate for endotoxin and ß(1,3)-glucan exposure assessment (Douwes *et al.*, 1996; Hyvarinen *et al.*, 2006). The dust samples from the homes of the 2-year-old children in the present study (Paper II) represent an overall exposure of the living areas in the house (living room, kitchen and bedrooms). Although this may be an appropriate surrogate marker for assessment of a child's overall exposure in the home, restricting the sampling area to e.g. the living room or bedroom would probably have been a better sampling procedure for the present study. Furthermore, the surface type such as carpets *vs* smooth floor and the presence of dust reservoirs like sofas, pillows, and soft toys may affect the concentrations of allergens and microbial agents (Arbes *et al.*, 2005; de Bilderling *et al.*, 2008).

It is generally accepted that air sampling is the most representative method to assess an individual's exposure to pet allergens by inhalation. However, the week-to-week variation of airborne Fel d1 (the major cat allergen) has been found to be substantial both in homes with and without cats due to varying degrees of disturbance by the humans and the cats (Bollinger *et al.*, 1996). Moreover, air sampling is time consuming and often impractical (because of noise). Settled dust is an attractive surrogate measure of personal exposure, in particular in epidemiological studies where the technique of measuring exposure must be simple, reliable, reproducible, and not time-consuming (Custovic *et al.*, 1999). Preferably, dust samples should be collected by fieldworkers with standardized procedures. However, comparisons of dust collection applied by study subjects and fieldworkers show that collection by study subjects are reliable (Arbes *et al.*, 2005; Schram-Bijkerk *et al.*, 2006). This procedure represents a practical option for allergen and microbial agent exposure assessment, due to both high return rates and moderate to high correlation to the levels of biological components collected by the fieldworkers (Arbes *et al.*, 2005; Schram-Bijkerk *et al.*, 2006).

Exposure to indoor moulds may vary by season due to outdoor variations in mould concentrations. Settled dust has also shown seasonal variation in levels of endotoxin (Hyvarinen *et al.*, 2006), HDM and cat allergens (Gehring *et al.*, 2001; Heinrich *et al.*, 2003). In our studies (Papers II and IV) we did not adjust for seasonal variation. Nevertheless, the

between-home variability due to housing type, pet ownership, and other household factors is usually greater than *within-home* variability in different seasons (Heinrich et al., 2003). Furthermore, repeated measurements of HDM allergen, cat allergen and endotoxin in settled house dust has been found to improve the estimate for annual mean concentrations. However, even a single observation of these biological contaminants may be a good proxy for the average annual exposure since repeated measures are usually highly correlated (Heinrich et al., 2003).

For the mattress dust sampled at the 10-year follow-up, we found that the amount (weight) of dust depended on the power of the vacuum cleaner the parents had used to collect the dust (results not shown). It was therefore not feasible to present the concentrations as $\mu g/m^2$, which instead was presented as µg/g dust. Using amounts of dust as a mean for hygiene (Gehring *et al.*, 2001) or concentrations of allergens and microbial components per square meter is nevertheless not recommended unless the sampling is strictly standardized (Wickens *et al.*, 2004).

6.2 Extraction and analysis of allergens, endotoxins and ß(1,3)-glucans

Dust samples may contain a broad spectrum of allergens, microbial agents and their components, proteolytic enzymes and other undefined substances. The dust samples are likely to vary in humidity depending on the conditions of the sampling locations. In un-frozen samples these factors could potentially lead to differences in post-sampling bacterial growth, but this is less likely to affect dust samples that are kept frozen until extraction and analysis, as was the case for the dust samples in the present study. The long storage time of the house dust samples (approximately 10 years) (Paper II) can potentially have affected the concentration of allergens and microbial components. Only Fel d1 (cat allergen) concentrations have been found to possibly decline with increasing storage time (up to 10 months), whereas, Der p1 (HDM), endotoxin and ß(1,3)-glucans were unaffected by the duration of storage (Fahlbusch *et al.*, 2003). Unfortunately, we do not know the effect of the storage time on our samples, but the levels of cat and dog allergens measured in homes with cat and dog keeping were significantly higher than in homes without these pets. However, it is mostly freeze-thaw cycles, and not the duration of storage that may affect the concentration levels (Douwes *et al.*, 1995; Fahlbusch *et al.*, 2003). All the samples in the present study have been treated in exactly the same way and have not been subjected to freeze-thawing cycles.

Also, the variation in allergen and microbial component levels between homes is probably much more pronounced than the potential influence of storage time (Fahlbusch *et al.*, 2003).

Unfortunately, we were not able to measure endotoxin and ß(1,3)-glucans in the mattress dust sampled at 10 years of age. These dust samples had been extracted for allergen analysis before the endotoxin and ß(1,3)-glucan extraction protocols had been established in our laboratory, and the extraction fluid was not endotoxin-free. The extraction of the floor dust samples (Paper II) for all analyses were performed by standardized protocols as established by the Institute for Risk Assessment Sciences (IRAS), Utrecht, the Netherlands (Schram-Bijkerk *et al.*, 2005). According to the protocol, the floor dust samples (Paper II) were not sieved, whereas the dust samples from the mattresses (Paper IV) were sieved. Sieving of dust has the advantage of removing heavy particles such as sand which may affect the weight of the sample. Thus, it would have been more appropriate to sieve the floor dust and not necessarily the mattress dust.

The conventional LAL assay that is used for endotoxin detection is not entirely specific for endotoxin and may give false-positive results with ß(1,3)-glucan (Vassallo and Limper, 1999; Zhang *et al.*, 1994). In the present study (Paper II) we did not find any correlation between the concentrations of ß(1,3)-glucan and endotoxin, but the microbial components were nevertheless mutually adjusted for each other as they were both included in the same multivariate analyses.

In the ELISAs used to measure the levels of allergens, the detection limits were determined according to the standard curve for each run. The levels below the detection limits were given a value of 2/3 of the detecting limit. For concentrations lying off the linear part of the standard curve, the assays were repeated with diluted samples. The results of the ELISA were given as the mean concentrations of the sample measured in two wells on the same plate, and a diluted sample may result in a poorer coefficient of variation between the wells compared to undiluted samples. Presenting variables in percentiles might mask the effect of the higher variability in the concentrations measured in diluted samples. However, the use of percentiles was not chosen because the resulting associations with health outcomes would then depend on the cut-off between the groups (see discussion of statistical methods below). Also, continuous variables will usually provide a better statistical power than using variables in categories.

6.3 Statistics

In Paper I all the 1019 children followed up to 10 years of age were included in the analyses. In the nested case-control group at 2 years of age, the controls were included as children *without* bronchial obstruction (BO) born closest in time to children *with* rBO during the first 2 years of life, and no other relevant matching was performed. However, analyses were not matched case-control analyses, as this would have lead to restricted sample-size for analyses. Although such analyses in general are optimal for subjects enrolled in a case-control study, the main aim of Paper I was to identify *which* factors could be important for pet avoidance and pet keeping without any a priori hypothesis of which variables this might be. Thus, all the predictor variables (including rBO or not) had the same 'status' in the multivariate models.

In Paper II, an alternative approach for analysing allergens, endotoxin and ß(1,3)-glucans would have been to categorize the exposure into groups using quartiles. Such an approach would, however, depend upon the cut-off value and important associations in the tail-ends (upper and lower) may be missed in the analyses. Moreover, using quartiles would only hamper the possibilities to compare ours with other results if different cut-off values are used. This is particularly important since our geographical area may have different overall levels of HDM allergens and endotoxin than found in other geographical areas and in rural communities.

Variables that are highly skewed, which is typical for cat and dog allergen concentrations measured in house dust, are often log-transformed. This is appropriate when applying parametric statistical methods. However, in the logistic regression model, the variables can be used as continuous variables without log-transformation, as long as the criterion concerning linearity is fulfilled. In Paper II, the condition was tested according to a procedure suggested by Sauerbrei and Royston (Sauerbrei and Royston, 2009). We performed a fractional polynomial analysis that supported treating the bio-contaminant variables as continuous and linear in the model (as the best non-linear transformations were not significantly different from the linear model). For the linear regression models, the condition of normally distributed residuals of the dependent variable was fulfilled in the models of FEV_1, FEF_{50} and change in lung function from birth to the age of 10 (Paper II).

In Paper II, we included a figure illustrating the predictive probability of current asthma based on risk associations (OR) from the logistic regression analysis (Figure 5). The x-axis of cat allergen exposure includes exposure levels from 0-800 µg/g dust. The majority of the children (95%) in the present study were exposed to cat allergen levels (Fel d1) below 100 µg/g dust. However, as the figure describes probabilities from 0 to 1, it will inevitably

include allergen levels that are above the levels that the children in the present study were exposed to at the age of 2 years.

In Paper II, an increased risk of current asthma at 10 years of age was found in relation to increasing exposure to cat allergens expressed in units of 10 µg/g dust. We could have expressed the risk of asthma by exposure to cat allergens in units of 1 µg/gram dust. The corresponding increase in adjusted odds ratio of asthma by 10 years would be 0.12. Thus, the conclusions remain the same independent of using a unit of 1 or 10 µg allergen/g dust. A change in 1 µg allergen/g dust is likely to be introduced as a day-to-day variation, whereas a unit of 10 µg allergen/g may be a better measure of between-home variability, but may also illustrate a difference between homes with and without pets.

The overall exposure to HDM allergen, Der p1, was in general low, and it was therefore more meaningful to use 1 µg as the unit of exposure for Der p1 (Paper IV). Furthermore, exposure levels measured in mattress dust are less prone to day-to-day variation and reporting the allergens in unit of 1 µg/g was therefore assumed to be more appropriate for the dust sampled from mattresses.

7 General discussion

7.1 Pet exposure and family history of allergic disease

In the present study, pet avoidance appeared to be a strong confounder for rhinitis, in particular for the rhinitis children with allergic sensitization. The majority of the children (89.6%) who were sensitized to inhalant allergens at 10 years of age had previously received a doctor-diagnosis of allergy to inhalant allergens. It is likely that these children had been assessed for allergic sensitization status due to some allergy-related symptoms. Confirmed allergic sensitization to inhalant allergens may thus have prevented the families from acquiring pets.

On the other hand, early asthma symptoms such as rBO during the first 2 years of life were not associated with *reported* or *actual* pet avoidance. Children with asthma-like symptoms early in life are at risk for developing asthma later in childhood (Martinez, 2009), as was found in the present cohort (Devulapalli *et al.*, 2008). Furthermore, a doctor's diagnosis of asthma (prior to the 10-year follow-up) did not influence the families' decision on pet removal or pet acquisition. However, only 34.6% of these children reported doctor-confirmed allergy to inhalant allergens, and avoiding pets may therefore not seem as important for 'non-allergic' types of asthma. However, the parents may not be aware of the risk of early asthma symptoms and ongoing allergic disease, or pet keeping may be more governed by social or emotional factors. Others have reported pet avoidance to be more likely for families of asthmatic children *with* rhinitis than for those *without* rhinitis (Hansel *et al.*, 2006), and that pet removal is commonly associated with disease development in the child (Bornehag *et al.*, 2003; Brunekreef *et al.*, 1992).

In the present study, atopic eczema in the index child was association with removal of dogs after birth, whereas cat removal was not associated with allergic disease in the index child or the child's family. Moreover, parental asthma and rhinoconjunctivitis was not associated with reduced pet keeping at the child's birth, or with pet removal or acquisition of pets after birth. Similarly, a report from the Dunedin cohort from New Zealand, found that parents with or without a history of asthma or hay fever were equally likely to have cats or dogs at the child's birth and to acquire cats or dogs during the first 9 years of life (Mandhane *et al.*, 2009).

Further, in the present study, only 3.4% of the children reported wheezing symptoms by contact with pets prior to the 10-year follow-up. However, at 10 years of age, 25% of the children suffered from current rhinitis, and these children were often allergically sensitized

and suffered from allergy-related co-morbidities including asthma, atopic eczema and conjunctivitis. Thus, pet removal may be more common with advancing age in this cohort since it is expected that more children will develop allergic disease or experience worsening of their symptoms. However, several studies have found that patients with asthma may rather take asthma medication to control their asthma, than getting rid of a beloved pet (Hansel *et al.*, 2006; Svanes *et al.*, 2006). This has also been observed for patients diagnosed with allergy to dogs or cats (Coren, 1997). Svanes *et al*. (2006) reported that families with asthmatic parents refrained from getting rid of the pet even when their child got asthma. On the other hand, pet *acquisition* was less common among subjects with asthma in the ECHRS study (Svanes *et al.*, 2006).

The families in the present cohort acquired other furry pets than cats or dogs regardless of allergic disease in any of the family members. Rodents may be perceived as being less allergenic than cats and dogs. Furthermore, it may also reflect the children's wish for pets as the gender of the index child (girl) and having older siblings were the strongest determinants for acquisition of other furry pets than cats or dogs.

At the time of the child's birth, only parental smoking and educational level were significantly associated with cat and dog keeping, respectively. Furthermore, smoking was associated with both cat and dog acquisition after birth. The associations between pet keeping and smoking as well as with a low parental education level have also been reported from the PIAMA birth cohort (Prevention and Incidence of Asthma and Mite Allergy) (Kerkhof *et al.*, 2009). Moreover, in the present study, single parenthood (n=246 with 78% single mothers) was associated with cat acquisition after the child's birth. Thus, it appears that factors associated with socio-economic status may be important predictors for pet ownership.

In the present study, the dog allergen levels in mattress dust at 10 years of age was lower for children *with* than without allergic sensitization, even though dog ownership was similar in the two groups. This could be due to a stronger effort to reduce the allergen load in families of sensitized children. Thus, avoidance may not only affect pet ownership, but also be expressed as *allergen* avoidance. Vacuuming is a common recommendation for allergen avoidance, but appears to have only minor effects on allergen removal (Tovey, 2008), although newer vacuum cleaners may be more efficient in removing allergens. In the present study, 10.0% of the families had central vacuum cleaners, but no association was seen between type of vacuum cleaner and allergens levels or reported pet avoidance (results not shown). Air cleaners have been recommended for reducing indoor allergens, and may be efficient in removing cat allergen, but not HDM allergen which is most often found in settled

dust reservoirs such as mattresses, pillows and sofas (Diette *et al.*, 2008; Tovey, 2008). Among the 1019 families in the present cohort, only 34 families reported to have air cleaners at home, and five families reported air cleaners in the child's bedroom. Thus, the numbers were too low to relate to allergen concentration. Another approach for reducing allergen exposure is to remove items that accumulate dust, such as carpets. The concentration of pet allergens may be as much as 100 times higher in carpets than on polished floors (Woodcock and Custovic, 1998). Floor carpeting is uncommon in Norway today compared to a few years ago. In the present study, 15.1% of the families had wall-to-wall carpeting in the living room during the child's first year of life compared to 2.4% at the 10-year follow-up. Similarly, 33.2% of the children had wall-to-wall carpeting on their bedroom floor during their first year of life and 10.5% at 10 years of age.

Among the families who *reported* pet avoidance at the 10-year follow-up, 22% had nevertheless currently at least one furry pet at home. Ten of these families had always kept pets and 58 families had acquired pets after the child's birth, of which 50% had acquired either a cat or a dog. The *reported* pet avoidance for these families is therefore likely to have been directed towards specific types of pets only, as also observed in the ECRHS study, where cats but not dogs were avoided (Svanes *et al.*, 2006).

Recall bias is always a hazard when doing analyses related to events occurring in the past. This was assessed at the 10-year follow-up, when the parents were asked questions regarding pet keeping during the child's first year of life and reports were compared to the 0-1 year questionnaires. At 10 years of age, 24% of the parents failed to report pets and 5% over-reported pets kept during the first year of life. Moreover, 33% of the parents who underreported pet ownership also reported pet avoidance in the same interview. In the PIAMA study, the parents were found to overstate the extent to which they avoided pets at home because of allergy in one of the family members (Kerkhof *et al.*, 2009). With both interviews and questionnaires, the subject's response to a question may be influenced by perceived expectations from the interviewer. However, incorrectly reported pet ownership in the present study was not associated with allergic disease in the family, in line with two other reports (Nicholas *et al.*, 2009; Svanes *et al.*, 2008).

The majority of those who underreported pet keeping in the present study had kept cats during the child's first year of life. A recent report on the accuracy of young adults to recall having childhood pets, found that both cats and dogs were more likely to be underreported than over-reported, and that recall of having cats and dogs was on average "moderate" (kappa statistics = 0.41-0.60) (Nicholas *et al.*, 2009). Svanes et al (2008) reported substantial

agreement between *adults* asked about their *childhood* pets on two separate occasions 9 years apart. Interestingly, in the PIAMA study, substantial disagreement between previous parental reports on doctor diagnosed asthma was found, when at the age of 8 years, as many as 40% of the children with a doctor's diagnosis of asthma according to prospective data were reported not to have had this diagnosis retrospectively (Kerkhof *et al.*, 2009). Thus, recall error of both disease and pet keeping can have consequences for the conclusions in studies assessing associations of pet keeping and allergic disease development.

7.2 Associations between pet exposure and allergic disease

In the present study we found that increased levels of cat allergens at 2 years of age increased the risk of current asthma at 10 years of age, but no association was seen with a history of asthma. Thus a group of children with a history of asthma did not exhibit symptoms or used asthma medication at 10 years of age, representing a phenotype that either suggest they have "outgrown" their asthma, that their phenotype is unrelated to pets or that they may be in a period of asthma remission. The 'window of opportunity' during which environmental influences affect the development of later childhood asthma has been suggested to be restricted to the prenatal, early postnatal or infant-young pre-school period (Carlsen *et al.*, 2009; Martinez, 2009), but the length of this period is uncertain. Assuming that cat allergen levels measured at 2 years of age are representative for the exposure levels the children experienced during the two first years of life, it may explain associations with asthma seen later in childhood. However, after stratifying for allergic sensitization, the association between increasing cat allergen levels at 2 years of age and the increasing risk of asthma at the age of 10 remained significant only for the children *without* allergic sensitization. Separating the risk for asthma and risk for sensitization is challenging, as allergic sensitization is a strong risk factor for asthma on it's own (Anto, 2004). In a long-term follow-up of children with early wheezing, early sensitization to cat and dog increased the risk of later asthma whereas early exposure to cat or dog had no such effect (Korppi *et al.*, 2008). However, reports on time trends in the development of allergic disease in children from the former East Germany have reported the prevalence of allergic sensitization also to increase for East German children who were born before the unification of East and West Germany, whereas the asthma prevalence increase only for children born after the unification (Heinrich *et al.*, 2002; von Mutius *et al.*, 1998). Thus, factors operating very early in life may be particularly important for the acquisition of childhood asthma, whereas the development of

allergic sensitization and hay fever may also be affected by environmental factors occurring beyond infancy.

In one population-based birth cohort study, associations between early life exposure to cat allergen and asthma at the age of 6 years was found, independent of allergic sensitization status (Torrent *et al.*, 2007). Interestingly, this study reports a steep increase in risk at low levels of exposure and a flattening above 1 µg/g of dust. It was argued that only for low levels of allergens different effects on asthma and sensitization may occur (Torrent *et al.*, 2007). This could be an additional explanation also for our findings and a possible explanation for the low adjusted odds ratio. Alternatively, allergens may possess an immunological and inflammatory mechanisms in addition to IgE responses. Results from studies on the proteolytic activity possessed by Der p1 have led to the suggestion that it would be more appropriate to consider allergens as polypeptides with a diverse range of biological effects, rather than proteins which simply trigger the synthesis of specific IgE (Chapman *et al.*, 2007; Wan *et al.*, 1999).

The children in cohort studies published so far tend to have short follow-up times (Apelberg *et al.*, 2001; Simpson and Custovic, 2005), and studies where the exposure is assessed shortly before outcome may show a protective effect in healthy children due to removal of the exposure in the diseased. Moreover, at very young age, too few children may have developed asthma and allergies during the follow-up to be able to detect any associations between exposure and disease development. The use of biologically meaningful timing of exposure and outcome is therefore of critical importance in studies of risk associations (Apelberg *et al.*, 2001; Chen and Heinrich, 2009).

In relation to exposure-disease associations for allergic diseases, much more attention has been given to cats than dogs in epidemiological studies (Simpson and Custovic, 2005). One reason may be publication bias due to more documented associations between cat allergen exposure and allergic disease development, since, in contrast, most respiratory symptoms related to dog exposure appear to be non-allergic (Langley *et al.*, 2005; Nafstad *et al.*, 2001; Svanes *et al.*, 1999; Svanes *et al.*, 2003). Thus, the major cat allergen Fel d1 is believed to be more allergenic than the major dog allergen Can f1 (Murray *et al.*, 1983). Fel d1 has also been found to have enzymatic activity (Wan *et al.*, 1999), although the nature of the enzymatic activity is not well known and weak compared to the enzymatic activity of Der p1. Moreover, Fel d1 belongs to the group of secretoglobulins, whereas Can f1, belongs to the family of lipocalins. The human body contains numerous endogenous lipocalins, and the immune system has had to adapt to their presence (Virtanen, 2001). As most T cells reactive

to lipocalins have been eliminated during thymic maturation, a weak cellular response (as observed with another lipocalin allergen, Bos d2 (cow)) could be due to the low frequency of T cells that are able to bind to Can f1 on allergen presenting cells (Mantyjarvi *et al.*, 2000). Another possibility is that the surviving T cells with their low-affinity T cell receptors for self, recognize allergen epitopes of lipocalin allergens inefficiently (Mantyjarvi *et al.*, 2000).

Studies assessing both cats and dogs, have often found that the exposure to cat allergens results in more severe symptoms than the effect of dog allergen exposure (Lindfors *et al.*, 1999; Murray *et al.*, 1983). One possible explanation, as mentioned above, is the nature of the Fel d1 allergen. Another explanation may be that cat allergens remain airborne for a longer period of time (Luczynska *et al.*, 1990), possibly resulting in higher inhaled doses of cat allergens. Airborne dust particles (mostly less than 2.5 μm in diameter) may act as carriers of allergens into the airways, and Fel d1, Can f1, and Bet v1 (birch allergen) have all been found to bind on the surface of airborne particles, in contrast to Der p1 (Ormstad *et al.*, 1995; Ormstad *et al.*, 1998). In addition, airborne particles may also act as an adjuvant causing inflammatory responses as well as increasing the production of sIgE (Samuelsen *et al.*, 2008).

In the present study, we did not find any association between cat and dog allergen levels at 2 years of age and specific sensitization to pet allergens later in life. An interesting finding was that the majority of children with sensitization to cat and dog at 10 years of age had never kept these pets at home (91% and 85%, respectively). These findings are in line with other studies where 70% or more of the cat allergic children had never lived in a house with a cat (Perzanowski *et al.*, 2002). This implies that low-level exposure to pet allergens is sufficient for susceptible individuals to become sensitized, or that they are exposed to much higher levels of pet allergens at other places than home (Almqvist *et al.*, 1999; Instanes *et al.*, 2005; Perzanowski *et al.*, 1999). In the Lifestyle-Immune-System-Allergy (LISA) birth cohort study, it was found that early life exposure to cat allergen increased the risk of allergic sensitization at 2 years of age, but found no association with allergic sensitization at the age of 6 (Chen *et al.*, 2007). On the other hand, *regular cat contact* without ownership increased the risk of sensitization to cat during childhood (at both age 2 and 6), but only for those children with parental allergic disease (Chen *et al.*, 2007).

It is likely that susceptible atopic individuals may become sensitized to allergens at lower concentrations than individuals that are less susceptible. This may explain the high prevalence of sensitization to cat and dog allergens among the children in the present study (figure 4) with additional sensitization to both seasonal and perennial allergens. Monosensitization is rather uncommon in susceptible atopic individuals, and is more likely to

occur in individuals exposed to extremely high levels of allergen and adjuvants, such as in occupational settings.

Cross-reactivity may contribute to the apparent multiple sensitizations. In general, patients sensitized to pets (particularly cats and dogs) frequently display IgE reactivity against allergenic proteins derived from different animals (Liccardi *et al.*, 2001). This suggests the possibility of cross-reactions between common allergenic determinants. In particular, the presence of a Fel d1-like allergen has been detected in dog dander extracts (Reininger *et al.*, 2007). It is therefore possible that some of the individuals with an elevated IgE apparently specific to Can f1 (dog), have actually been sensitized to cats rather than dogs.

Cat and dog allergen levels in mattress dust at 10 years of age were not associated with current symptoms of rhinitis, as these children were more likely to avoid pets and to have lower allergen levels in their mattress dust compared to children without allergic rhinitis. However, the children who were unable to relate any triggers to their symptoms had higher levels of dog allergens in their mattress dust compared to the children who reported at least one perceived rhinitis trigger. Although this association is of uncertain importance as it was not confirmed in the multivariate analyses, few of these children kept dogs and may be unaware of the exposure at home, possibly due to transfer of allergens on clothes from contact with the allergens elsewhere.

7.3 The influence of exposure to house dust mite and microbial components on allergic disease

In the present study, the overall exposure to HDM allergen was low. Also in mattress dust where the HDM allergen is most common (Custovic *et al.*, 1999), detectable levels were demonstrated in only 12.3% of the samples. This is consistent with earlier findings in the ECA study with Der p1 allergens measured in mattress dust of the 2-year-old children included in the nested case-control group (Nafstad *et al.*, 1998), and with other previous reports from Norway (Dotterud *et al.*, 1995; Dotterud *et al.*, 1997). The HDM allergen is contained in relatively large mite feces particles, typically 10-40 μm in diameter, and is detected in the air of indoor environments only after a strong disturbance (Custovic *et al.*, 1999; Liccardi *et al.*, 2001). Under undisturbed conditions, HDM allergen may be found only in dust reservoirs, such as mattresses, pillows and carpets. Because of the large size, the mite feces particles are generally considered non-respirable. Some large particles may enter the lung, but the majority are usually cleared very rapidly (Svartengren *et al.*, 1987). However, in

regard to sensitization to allergens, this is likely to occur in the mucosa linings of the nose and nasopharynx.

In one previous study from Norway, the few homes with high mite allergen levels were homes with poor ventilation, increased humidity and water leakages (Dotterud *et al.*, 1995). No such associations were found in the present study (results not shown), and in general few homes reported problems with water leakages (4.4%) or dampness (5.9%) at the 10-year follow-up.

Nevertheless, children with Der p1 sensitization still had higher levels of Der p1 allergen in their mattress dust compared to children without Der p1 sensitization. Similar results have also been found in other studies from Norway (Dotterud *et al.*, 1995; Dotterud *et al.*, 1997) and from Sweden (Warner *et al.*, 1996). Our findings are in conformity with the notion that the Der p1 allergen is more potent than cat and dog allergens in relation to sensitization and allergic inflammation development, possibly due to the intrinsic enzymatic activity of Der p1 (Liccardi *et al.*, 2001). Der p1 have several potent effects on the epithelium, such as cleavage of proteins of tight junctions resulting in paracellular delivery of allergens (Wan *et al.*, 1999). Further, Der p1 may act as Th2 cell adjuvant by cleaving CD23 from activated B cells and CD25 from T cells (Chapman *et al.*, 2007). Another explanation for allergic sensitization in individuals, who are not exposed to HDM allergen at home, may be exposure to the allergen during holidays or other visits to areas where HDM is more prevalent (Hallas *et al.*, 2004; Wickman *et al.*, 1993).

In contrast to other birth cohort studies (Celedon *et al.*, 2007; Douwes *et al.*, 2006; Gillespie *et al.*, 2006), we found no evidence of modifying effects of endotoxin in house dust, except for a weak association with FEV_1 % predicted at 10 years of age, which disappeared after removing one extreme outlier. In the study of Celedon et al. (2007) early life exposure to endotoxin above the lowest quartile was found to be associated with a reduced risk of atopy at school-age, but with an increased risk of wheeze between 1 and 7 years of age. However, even the *lowest* quartile in that study was above the levels of endotoxin measured in the homes of the 2-year-old children in the present study. Moreover, the protective effect of endotoxin exposure has most often been reported in farming households where exposure is likely to occur throughout pregnancy, early post-natal life and throughout infancy, and with much higher endotoxin levels than in non-farming households (Liu, 2004). Genetic variation in toll-like receptors, TLR2 and TLR4, has also been suggested to be an important determinant of the susceptibility to asthma and allergies in children of farmers and in relation to high endotoxin exposure, respectively (Eder *et al.*, 2004). Furthermore, the birth cohort

studies reporting associations with endotoxin and allergic disease outcomes were all with high-risk children and with shorter follow-up time than in the present study (Celedon *et al.*, 2007; Douwes *et al.*, 2006; Gillespie *et al.*, 2006). Notably, the association between endotoxin exposure and reported airway and skin symptoms in 15 months old children, as reported by Gillespie *et al.* (2006), was with only 12 months follow-up time between exposure and outcome assessment. Thus, the effect of endotoxin exposure on allergic disease development appears to be influenced by the timing of exposure, pre-existing disease and gene-environment interactions (Williams *et al.*, 2005).

The paradox that high levels of endotoxin induce asthma-like symptoms is mostly observed in an occupational setting (Liu, 2004), and may with continuous exposure lead to chronic airway diseases (Schwartz *et al.*, 1995). The present study was not designed to assess the effect of continous exposure, and the tendency towards decreased FEV_1% predicted at 10 years of age with increasing endotoxin levels at 2 years of age can not be attributed to continued exposure.

ß(1,3)-glucans may, like endotoxin, possess inflammatory and adjuvant effects (Novak and Vetvicka, 2008; Ormstad *et al.*, 2000). In the present study, we found no association between levels of ß(1,3)-glucans at 2 years of age and allergic disease outcomes at 10 years of age, which is in line with another prospective birth cohort study assessing asthma, wheeze and allergic sensitization in 4-year-old children (Douwes *et al.*, 2006). Concurrent exposure to ß(1,3)-glucans have been found to protect against wheezing in farm children (Iossifova *et al.*, 2007; Schram-Bijkerk *et al.*, 2005), where the levels of ß(1,3)-glucans were found to be increased (Schram *et al.*, 2005). Thus, although ß(1,3)-glucans may have immunomodulatory effects, the evidence of a causal role for ß(1,3)-glucan in relation to asthma and sensitization in humans is still very limited.

The presence of pets may be associated with both ß(1,3)-glucan and endotoxin levels in house dust (Gehring *et al.*, 2001; Gehring *et al.*, 2004), but in the present study we found no association between pet keeping and the levels of endotoxin or ß(1,3)-glucans.

7.4 Gender in relation to pet exposure

In the present study, the 10-year-old boys were more likely than the girls to have current asthma, rhinitis, and wheeze and to be allergically sensitized. Sensitization to cat allergen was also more common for boys (16.2%) than for girls (8.6%), p=0.001, which may explain why boys had less cats at home and were in less contact with cats outside home compared to the girls, although not significantly so when assessing cat ownership separately. Boys were also

more often sensitized to dog allergen compared to girls (18.3% and 8.1%, respectively, $p<0.001$), but no difference between the genders was seen in regard to dog ownership or contact. In general, sensitized children appeared to be more likely to avoid cats than dogs, which may be due to the symptoms caused by cat allergen appear to be more severe compared to symptoms induced by dog allergen (Murray *et al.*, 1983). The children may also have a false confirmed allergy test to dog allergen, as previously discussed, and may therefore be less likely to have clinical symptoms when near dogs. On the other hand, the families with other furry pets than cats and dogs had also more often daughters than sons, and cat ownership *at birth* was also more common in families where the index child was a girl (results not shown). Although this is likely to be a statistical coincidental finding at birth, girls favouring furry pets might contribute to the association between female gender and pet keeping at 10 years of age. Single parenthood was also a significant predictor for cat acquisition after the child's birth, and of these 254 single parents, 78% were single mothers, indicating that also female adults are more inclined to keep cats.

The association between female gender and pets was further supported by pet allergens in mattress dust being significantly higher for girls than for boys, regardless of pet ownership. In a recent Swedish study more cat keeping was found among girls than boys, with a significant interactive effect on the risk for current wheeze (Bjerg *et al.*, 2009). One longitudinal study on adults, found female smokers with household pets to be more likely to develop asthma during the two-year study period, compared to their male counterparts (Chen *et al.*, 2002). Moreover, in a previous report from the nested case-control group from the ECA-study, gender differences were seen for antenatal maternal smoking and sCD14 levels (Lodrup Carlsen *et al.*, 2006b). This supports the hypothesis that environmental factors influence the immune system differently in girls and boys, as also proposed by Ronchetti *et al.* (1992) who reported parental smoking to enhance allergic sensitization in boys, but not in girls (Ronchetti *et al.*, 1992).

In spite of the difference in allergic diseases in boys and girls and the common knowledge that this is not due to biological differences alone, surprisingly few studies have assessed differences in exposure between genders. In most studies gender effects are recognized and adjusted for in analyses assessing associations between risk factors and allergic disease development, thus masking gender as a possible important explanatory factor. To our knowledge, the present study (Paper IV) is the first to report differences in indoor allergen levels between boys and girls. However, due to the effect of *reported* pet avoidance

on pet keeping for the children with allergic rhinitis, no gender related differences were observed between pet allergen levels and current rhinitis in the multivariate models.

Interestingly, HDM allergen significantly increased the risk of current rhinitis in girls, but not in boys. For boys, the association with HDM allergen levels and current rhinitis symptoms may be masked due to the strong association between current asthma and current rhinitis. Our findings imply that even low levels of HDM allergen in mattress dust may be associated with induction of rhinitis symptoms, as well as with allergic sensitization, as previously discussed.

7.5 Strengths and limitations

The ECA study is a prospective birth cohort study which was designed to investigate asthma and factors associated with asthma development during childhood, with emphasis on environmental exposures. It contains a well characterized population of mainly, but not exclusively, Norwegian ethnicity. The half-yearly prospective questionnaires during the two first years of life reduce the risk of recall bias and misclassification thereby increasing the level of evidence. The 84% participation rate at 10 years of age is high and few other European birth cohorts have, so far, had such a long follow-up time (Keil *et al.*, 2006). The high participation rate also reduces the risk for inclusion bias.

At 10 years of age the definition of asthma ever was based on a *combination* of symptoms, medication and clinical diagnosis. Current asthma included the objective measure of airway bronchial hyperresponsiveness by treadmill running. These definitions reduce the risk of misclassification thereby increasing the validity of our findings. Further, we included the core questions from the standardized ISAAC questionnaires, making it possible to compare our results with other studies.

The clinical examination of all children was performed by study doctors with experience in paediatric pulmonology and allergology, and all staff involved in the 10-year follow-up study were blinded to the results of the lung function measurements at birth as well as to all other previous results.

The 10-year follow-up study is asthma-enriched due to the inclusion of the nested case-control group (at 2 years of age), where the cases had rBO. As previously discussed, this was either corrected for by weighted prevalence analyses (Paper III) or with rBO included as a potential explanatory variable on its own (Paper I). The *concurrent* allergen levels and rhinitis symptoms assessed in Paper IV, were probably unrelated to rBO status during the two first years of life. rBO was already confirmed not to be associated with pet avoidance (and

thus correspondingly lower pet allergen levels), and the intention of the study was nevertheless to study the association between concurrent exposure and rhinitis symptoms and *not* early life risk factors of rhinitis.

Cohen's kappa (κ) (Landis and Koch, 1977) was used to assess the agreement between SPT and sIgE antibody > 0.35kU/L specific for cat and dog allergens for the children included in the 10-year follow-up study. The agreement between SPT and sIgE was $\kappa = 0.82$ for cat and $\kappa = 0.60$ for dog allergen. According to Landis & Koch (1977) 0.60 - 0.80 represent a substantial agreement and 0.80 - 1.00 an almost perfect agreement. The lower agreement between SPT and sIgE antibodies for dog allergen may be due to the Fel d1-like allergen in the dog dander extract used for detection of sIgE to dog (Reininger et al., 2007). Our results might have been different if allergic sensitization to a specific allergen had been defined by both a positive sIgE *and* SPT, or by either of these alone. However, our outcomes were most often related to sensitization to any inhalant allergen and the majority of the children were nevertheless sensitized to more than one allergen.

7.6 Future perspectives

The results of the papers included in the present thesis raise some important questions. First, how do pet keeping during the first years of life and throughout childhood affect the development of allergic disease later in adolescence? By following children included in the birth cohort into adolescence, we may use prospectively collected information on allergen levels, pet removal and pet acquisition and see how this relates to development of allergic disease later in life. Further, considering our finding that few families removed their pets, it would be of interest to assess how allergic diseases at 10 years of age affected pet removal later in childhood or adolescent.

Studies assessing microbial substances and allergens in house dust have often given conflicting results in relation to exposure-effect associations, and also the amount of dust alone appears to affect the development of allergic diseases (Gehring et al., 2007). Thus, factors that we so far do not fully comprehend may interact with these exposure-effect associations, or the microbial substances measured in house dust may be surrogate markers for something else. The second question is therefore whether there are other agents in the dust samples than may affect the development of allergic disease? One possibility is pollen and food allergens. Although both allergens have been measured in indoor dust reservoirs (Dotterud *et al.*, 1997; Fahlbusch *et al.*, 2001), few studies have assessed the association with food and pollen allergen levels in house dust and the development or exacerbation of allergic

diseases. In addition to biological factors, chemical factors such as phthalates may also affect the immune system, and can be quantified in dust samples and also be measured as biomarkers in urine. It has been of growing concern that everyday exposure to these and other chemicals through food, water and air may have impact on immune diseases, like asthma and allergy. However, the level of exposure in Norwegian children is not well known, and there are so far only a few epidemiological studies that have investigated the association between the so called 'modern' chemicals and allergic diseases.

Thirdly, it would be of interest to study how factors in the indoor environment contribute to allergen levels in the indoor environment, such as ventilation (mechanical *vs* natural), dampness, and different types of dwellings. Finally, further studies on differences in exposure and effects between boys and girls appear warranted and might also shed some light on the mechanisms involved.

8 CONCLUSIONS

Based upon the results from the present work concerning children included in the ECA study in Oslo and previous knowledge in the research areas as discussed in the present thesis, the following conclusion can be drawn on the specific research questions:

1. The results from the present work show that allergic disease in the family did not significantly decrease the likelihood of pet keeping at the child's birth. However, when the index child had allergic rhinitis, the families often avoided keeping cats and dogs. *Reported* pet avoidance appeared to be most common in families where the parents had high education, whereas pet removal even after appearance of symptoms of allergic disease, was uncommon in the present cohort.

2. In a sub-group of children from the ECA-study, early life exposure to cat allergen was found to increase the risk of asthma at 10 years of age, but no association was seen between the levels of pet allergens in the home at 2 years of age and allergic sensitization at 10 years of age. Cat and dog allergen levels in mattress dust at 10 years of age were not associated with current symptoms of rhinitis.

3. House dust mite allergen levels measured in homes in Oslo were generally low, but children who were sensitized to house dust mite allergen, Der p1, had significantly higher levels of allergens in their mattress dust than those who were not sensitized to Der p1. Endotoxin and ß(1,3)-glucan levels in house dust at 2 years of age did not modify the allergen or pet-related risk of allergic disease or lung function at 10 years of age.

4. Girls were more likely to keep furry pets and were also exposed to higher levels of cat and dog allergens at 10 years of age. However, in the multivariate analyses, house dust mite, but not cat and dog allergen levels, were associated with an increased risk of current rhinitis, but this was observed among girls only.

9 References

Abbas AK and Lichtman AH (2006) *Basic immunology: function and disorders of the immune system*, 2 ed. Philadelphia, PA, Saunders, Elsevier Inc.

Abraham JH, Finn PW, Milton DK, et al. (2005) Infant home endotoxin is associated with reduced allergen-stimulated lymphocyte proliferation and IL-13 production in childhood. *J Allergy Clin Immunol*, **116**, 431-437.

Alfredo MJ, Enriquez L, Moreno-Aliaga MJ, et al. (2007) Genetics of obesity. *Public Health Nutr*, **10**, 1138-1144.

Almqvist C, Egmar AC, Hedlin G, et al. (2003) Direct and indirect exposure to pets - risk of sensitization and asthma at 4 years in a birth cohort. *Clin Exp Allergy*, **33**, 1190-1197.

Almqvist C, Larsson PH, Egmar AC, et al. (1999) School as a risk environment for children allergic to cats and a site for transfer of cat allergen to homes. *J Allergy Clin Immunol*, **103**, 1012-1017.

Almqvist C, Pershagen G, Wickman M (2005) Low socioeconomic status as a risk factor for asthma, rhinitis and sensitization at 4 years in a birth cohort. *Clin Exp Allergy*, **35**, 612-618.

Almqvist C, Worm M, Leynaert B (2008) Impact of gender on asthma in childhood and adolescence: a GA2LEN review. *Allergy*, **63**, 47-57.

Anto JM (2004) The causes of asthma: the need to look at the data with different eyes. *Allergy*, **59**, 121-123.

Apelberg BJ, Aoki Y, Jaakkola JJ (2001) Systematic review: Exposure to pets and risk of asthma and asthma-like symptoms. *J Allergy Clin Immunol*, **107**, 455-460.

Arbes SJ, Sever M, Vaughn B, et al. (2005) Feasibility of using subject-collected dust samples in epidemiologic and clinical studies of indoor allergens. *Environ Health Perspect*, **113**, 665-669.

Asarnoj A, Ostblom E, Kull I, et al. (2008) Sensitization to inhalant allergens between 4 and 8 years of age is a dynamic process: results from the BAMSE birth cohort. *Clin Exp Allergy*, **38**, 1507-1513.

Asher MI, Montefort S, Bjorksten B, et al. (2006) Worldwide time trends in the prevalence of symptoms of asthma, allergic rhinoconjunctivitis, and eczema in childhood: ISAAC Phases One and Three repeat multicountry cross-sectional surveys. *Lancet*, **368**, 733-743.

Bakirci N, Kalaca S, Francis H, et al. (2007) Natural history and risk factors of early respiratory responses to exposure to cotton dust in newly exposed workers. *J Occup Environ Med*, **49**, 853-861.

Bakken HN, Nafstad P, Bolle R, et al. (2007) Skin sensitization in school children in northern and southern Norway. *J Asthma*, **44**, 23-27.

Barraclough R, Devereux G, Hendrick DJ, et al. (2002) Apparent but not real increase in asthma prevalence during the 1990s. *Eur Respir J*, **20**, 826-833.

Becklake MR and Kauffmann F (1999) Gender differences in airway behaviour over the human life span. *Thorax*, **54**, 1119-1138.

Belanger K, Beckett W, Triche E, et al. (2003) Symptoms of wheeze and persistent cough in the first year of life: associations with indoor allergens, air contaminants, and maternal history of asthma. *Am J Epidemiol*, **158**, 195-202.

Berntsen S, Carlsen KC, Anderssen SA, et al. (2009) Norwegian adolescents with asthma are physical active and fit. *Allergy*, **64**, 421-426.

Bischof W, Koch A, Gehring U, et al. (2002) Predictors of high endotoxin concentrations in the settled dust of German homes. *Indoor Air*, **12**, 2-9.

Bjerg A, Sandstrom T, Lundback B, et al. (2009) Time trends in asthma and wheeze in Swedish children 1996-2006: prevalence and risk factors by sex. *Allergy*, doi: 10.1111/j.1398-9995.2009.02105.x

Blackley C.H. (1988) *Experimental Researches on the Causes and Nature of Catarrhus Aestivus (Facsimile of the first edition 1873)* Oxford, Oxford Historical Books.

Bodtger U, Poulsen LK, Linneberg A (2006) Rhinitis symptoms and IgE sensitization as risk factors for development of later allergic rhinitis in adults. *Allergy*, **61**, 712-716.

Bollinger ME, Eggleston PA, Flanagan E, et al. (1996) Cat antigen in homes with and without cats may induce allergic symptoms. *J Allergy Clin Immunol*, **97**, 907-914.

Bornehag CG, Blomquist G, Gyntelberg F, et al. (2001) Dampness in buildings and health. Nordic interdisciplinary review of the scientific evidence on associations between exposure to "dampness" in buildings and health effects (NORDDAMP). *Indoor Air*, **11**, 72-86.

Bornehag CG, Sundell J, Bonini S, et al. (2004) Dampness in buildings as a risk factor for health effects, EUROEXPO: a multidisciplinary review of the literature (1998-2000) on dampness and mite exposure in buildings and health effects. *Indoor Air*, **14**, 243-257.

Bornehag CG, Sundell J, Hagerhed L, et al. (2003) Pet-keeping in early childhood and airway, nose and skin symptoms later in life. *Allergy*, **58**, 939-944.

Bornehag CG, Sundell J, Hagerhed-Engman L, et al. (2005) Association between ventilation rates in 390 Swedish homes and allergic symptoms in children. *Indoor Air*, **15**, 275-280.

Bousquet PJ, Burbach G, Heinzerling LM, et al. (2009) GALEN skin test study III: Minimum battery of test inhalent allergens needed in epidemiological studies in patients. *Allergy*, **64**, 1656-1662.

Braback L and Forsberg B (2009) Does traffic exhaust contribute to the development of asthma and allergic sensitization in children: findings from recent cohort studies. *Environ Health*, doi: 10.1186/1476-069X-8-17

Braback L, Hjern A, Rasmussen F (2005) Social class in asthma and allergic rhinitis: a national cohort study over three decades. *Eur Respir J*, **26**, 1064-1068.

Braback L, Kjellman NI, Sandin A, et al. (2001) Atopy among schoolchildren in northern and southern Sweden in relation to pet ownership and early life events. *Pediatr Allergy Immunol*, **12**, 4-10.

Braman SS (2006) The global burden of asthma. *Chest*, **130**, 4S-12S.

Brasche S and Bischof W (2005) Daily time spent indoors in German homes--baseline data for the assessment of indoor exposure of German occupants. *Int J Hyg Environ Health*, **208**, 247-253.

Bruce S, Nyberg F, Melen E, et al. (2009) The protective effect of farm animal exposure on childhood allergy is modified by NPSR1 polymorphisms. *J Med Genet*, **46**, 159-167.

Brunekreef B, Groot B, Hoek G (1992) Pets, allergy and respiratory symptoms in children. *Int J Epidemiol*, **21**, 338-342.

Brunetti L, Colazzo D, Francavilla R, et al. (2007) The role of pulmonary infection in pediatric asthma. *Allergy Asthma Proc*, **28**, 190-193.

Brussee JE, Smit HA, van Strien RT, et al. (2005) Allergen exposure in infancy and the development of sensitization, wheeze, and asthma at 4 years. *J Allergy Clin Immunol*, **115**, 946-952.

Burgess JA, Lowe AJ, Matheson MC, et al. (2009) Does eczema lead to asthma? *J Asthma*, **46**, 429-436.

Burney P, Malmberg E, Chinn S, et al. (1997) The distribution of total and specific serum IgE in the European Community Respiratory Health Survey. *J Allergy Clin Immunol*, **99**, 314-322.

Carlsen KC, Haland G, Carlsen KH (2009) Natural history of lung function in health and diseases. *Curr Opin Allergy Clin Immunol*, **9**, 146-150.

Carlsen KH and Carlsen KC (2008) Respiratory effects of tobacco smoking on infants and young children. *Paediatr Respir Rev*, **9**, 11-19.

Carlsen KH, Engh G, Mork M (2000) Exercise-induced bronchoconstriction depends on exercise load. *Respir Med*, **94**, 750-755.

Celedon JC, Milton DK, Ramsey CD, et al. (2007) Exposure to dust mite allergen and endotoxin in early life and asthma and atopy in childhood. *J Allergy Clin Immunol*, **120**, 144-149.

Chapman MD, Heymann PW, Wilkins SR, et al. (1987) Monoclonal immunoassays for major dust mite (Dermatophagoides) allergens, Der p I and Der f I, and quantitative analysis of the allergen content of mite and house dust extracts. *J Allergy Clin Immunol*, **80**, 184-194.

Chapman MD, Wunschmann S, Pomes A (2007) Proteases as Th2 adjuvants. *Curr Allergy Asthma Rep*, **7**, 363-367.

Chen CM and Heinrich J (2009) Re: Exposure to furry pets and the risk of asthma and allergic rhinitis: a meta-analysis. *Allergy*, **64**, 494-495.

Chen CM, Rzehak P, Zutavern A, et al. (2007) Longitudinal study on cat allergen exposure and the development of allergy in young children. *J Allergy Clin Immunol*, **119**, 1148-1155.

Chen Y, Dales R, Tang M, et al. (2002) Sex-related interactive effect of smoking and household pets on asthma incidence. *Eur Respir J*, **20**, 1162-1166.

Ciprandi G, Cirillo I, Vizzaccaro A, et al. (2005) Seasonal and perennial allergic rhinitis: is this classification adherent to real life? *Allergy*, **60**, 882-887.

Claussen O (1948) Asthma prevalence among school children in Norway. *Nord Med*, **37**, 525-530.

Cole JC, Ownby DR, Havstad SL, et al. (2004) Family history, dust mite exposure in early childhood, and risk for pediatric atopy and asthma. *J Allergy Clin Immunol*, **114**, 105-110.

Coren S (1997) Allergic patients do not comply with doctors' advice to stop owning pets. *BMJ*, **314**, 517.

Crapo RO, Casaburi R, Coates AL, et al. (2000) Guidelines for methacholine and exercise challenge testing-1999. This official statement of the American Thoracic Society was adopted by the ATS Board of Directors, July 1999. *Am J Respir Crit Care Med*, **161**, 309-329.

Crestani E, Guerra S, Wright AL, et al. (2004) Parental asthma as a risk factor for the development of early skin test sensitization in children. *J Allergy Clin Immunol*, **113**, 284-290.

Cruz AA, Popov T, Pawankar R, et al. (2007) Common characteristics of upper and lower airways in rhinitis and asthma: ARIA update, in collaboration with GA(2)LEN. *Allergy*, **62 Suppl 84**, 1-41.

Cullinan P, MacNeill SJ, Harris JM, et al. (2004) Early allergen exposure, skin prick responses, and atopic wheeze at age 5 in English children: a cohort study. *Thorax*, **59**, 855-861.

Custovic A, Simpson A, Woodcock A (1998) Importance of indoor allergens in the induction of allergy and elicitation of allergic disease. *Allergy*, **53**, 115-120.

Custovic A, Simpson B, Simpson A, et al. (1999) Relationship between mite, cat, and dog allergens in reservoir dust and ambient air. *Allergy*, **54**, 612-616.

D'Amato G, Liccardi G, Russo M, et al. (1997) Clothing is a carrier of cat allergens. *J Allergy Clin Immunol*, **99**, 577-578.

de Bilderling G, Mathot M, Agustsson S, et al. (2008) Early skin sensitization to aeroallergens. *Clin Exp Allergy*, **38**, 643-648.

Delves PJ and Roitt IM (2000) The immune system. First of two parts. *N Engl J Med*, **343**, 37-49.

Devulapalli CS, Carlsen KC, Haland G, et al. (2008) Severity of obstructive airways disease by age 2 years predicts asthma at 10 years of age. *Thorax*, **63**, 8-13.

Diette GB, McCormack MC, Hansel NN, et al. (2008) Environmental issues in managing asthma. *Respir Care*, **53**, 602-615.

Doll R and Hill AB (1952) A study of the aetiology of carcinoma of the lung. *Br Med J*, **2**, 1271-1286.

Dotterud LK, Korsgaard J, Falk ES (1995) House-dust mite content in mattresses in relation to residential characteristics and symptoms in atopic and nonatopic children living in northern Norway. *Allergy*, **50**, 788-793.

Dotterud LK, Van TD, Kvammen B, et al. (1997) Allergen content in dust from homes and schools in northern Norway in relation to sensitization and allergy symptoms in schoolchildren. *Clin Exp Allergy*, **27**, 252-261.

Douwes J (2005) (1-->3)-Beta-D-glucans and respiratory health: a review of the scientific evidence. *Indoor Air*, **15**, 160-169.

Douwes J, Doekes G, Montijn R, et al. (1996) Measurement of beta(1-->3)-glucans in occupational and home environments with an inhibition enzyme immunoassay. *Appl Environ Microbiol*, **62**, 3176-3182.

Douwes J and Pearce N (2003) Invited commentary: is indoor mold exposure a risk factor for asthma? *Am J Epidemiol*, **158**, 203-206.

Douwes J, van der SB, Doekes G, et al. (1999) Fungal extracellular polysaccharides in house dust as a marker for exposure to fungi: relations with culturable fungi, reported home dampness, and respiratory symptoms. *J Allergy Clin Immunol*, **103**, 494-500.

Douwes J, van SR, Doekes G, et al. (2006) Does early indoor microbial exposure reduce the risk of asthma? The Prevention and Incidence of Asthma and Mite Allergy birth cohort study. *J Allergy Clin Immunol*, **117**, 1067-1073.

Douwes J, Versloot P, Hollander A, et al. (1995) Influence of Various Dust Sampling and Extraction Methods on the Measurement of Airborne Endotoxin. *Appl Environ Microbiol*, **61**, 1763-1769.

Dreborg S, Einarsson R, Lau S, et al. (1995) Dust sampling for determination of allergen content. *Allergy*, **50**, 188-189.

Dreborg S and Frew A (1993) Position Paper - Allergen Standardization and Skin-Tests. *Allergy*, **48**, 49-82.

Eder W, Ege MJ, von ME (2006) The asthma epidemic. *N Engl J Med*, **355**, 2226-2235.

Eder W, Klimecki W, Yu L, et al. (2004) Toll-like receptor 2 as a major gene for asthma in children of European farmers. *J Allergy Clin Immunol*, **113**, 482-488.

Ege MJ, Frei R, Bieli C, et al. (2007) Not all farming environments protect against the development of asthma and wheeze in children. *J Allergy Clin Immunol*, **119**, 1140-1147.

Eichler I, Gotz M, Jarisch R, et al. (1988) Reproducibility of skin prick testing with allergen extracts from different manufacturers. *Allergy*, **43**, 458-463.

Eller E, Roll S, Chen CM, et al. (2008) Meta-analysis of determinants for pet ownership in 12 European birth cohorts on asthma and allergies: a GA2LEN initiative. *Allergy*, **63**, 1491-1498.

Ellison-Loschmann L, Sunyer J, Plana E, et al. (2007) Socioeconomic status, asthma and chronic bronchitis in a large community-based study. *Eur Respir J*, **29**, 897-905.

Fahlbusch B, Hornung D, Heinrich J, et al. (2001) Predictors of group 5 grass-pollen allergens in settled house dust: comparison between pollination and nonpollination seasons. *Allergy*, **56**, 1081-1086.

Fahlbusch B, Koch A, Douwes J, et al. (2003) The effect of storage on allergen and microbial agent levels in frozen house dust. *Allergy*, **58**, 150-153.

Flaherman V and Rutherford GW (2006) A meta-analysis of the effect of high weight on asthma. *Arch Dis Child*, **91**, 334-339.

Fokkens WJ (2002) Thoughts on the pathophysiology of nonallergic rhinitis. *Curr Allergy Asthma Rep*, **2**, 203-209.

Gehring U, Bischof W, Borte M, et al. (2004) Levels and predictors of endotoxin in mattress dust samples from East and West German homes. *Indoor Air*, **14**, 284-292.

Gehring U, Douwes J, Doekes G, et al. (2001) beta(1 -> 3)-glucan in house dust of German homes: Housing characteristics, occupant behavior, and relations with endotoxins, allergens, and molds. *Environ Health Perspect*, **109**, 139-144.

Gehring U, Heinrich J, Hoek G, et al. (2007) Bacteria and mould components in house dust and children's allergic sensitisation. *Eur Respir J*, **29**, 1144-1153.

Gillespie J, Wickens K, Siebers R, et al. (2006) Endotoxin exposure, wheezing, and rash in infancy in a New Zealand birth cohort. *J Allergy Clin Immunol*, **118**, 1265-1270.

Gilmour MI, Jaakkola MS, London SJ, et al. (2006) How exposure to environmental tobacco smoke, outdoor air pollutants, and increased pollen burdens influences the incidence of asthma. *Environ Health Perspect*, **114**, 627-633.

Giovannangelo M, Gehring U, Nordling E, et al. (2007) Determinants of house dust endotoxin in three European countries - the AIRALLERG study. *Indoor Air*, **17**, 70-79.

Glue C, Platzer MH, Larsen ST, et al. (2005) Phthalates potentiate the response of allergic effector cells. *Basic Clin Pharmacol Toxicol*, **96**, 140-142.

Gold DR, Wang X, Wypij D, et al. (1996) Effects of cigarette smoking on lung function in adolescent boys and girls. *N Engl J Med*, **335**, 931-937.

Gordis L (2000) *Epidemiology*, 2 ed. Pennsylvania, W.B. Saunders Company.

Gould HJ and Sutton BJ (2008) IgE in allergy and asthma today. *Nat Rev Immunol*, **8**, 205-217.

Gustafsson D, Sjoberg O, Foucard T (2000) Development of allergies and asthma in infants and young children with atopic dermatitis--a prospective follow-up to 7 years of age. *Allergy*, **55**, 240-245.

Haland G, Lodrup Carlsen KC, Mowinckel P, et al. (2009) Lung function at 10 yr is not impaired by early childhood lower respiratory tract infections. *Pediatr Allergy Immunol*, **20**, 254-260.

Hallas TE, Gislason D, Bjornsdottir US, et al. (2004) Sensitization to house dust mites in Reykjavik, Iceland, in the absence of domestic exposure to mites. *Allergy*, **59**, 515-519.

Hamilton L (1991) *Regression with Graphics: A Second Course in Applied Statistics.* Pacific Grove, California, Brooks/Cole Publishing Company.

Hansel NN, Eggleston PA, Krishnan JA, et al. (2006) Asthma-related health status determinants of environmental control practices for inner-city preschool children. *Ann Allergy Asthma Immunol*, **97**, 409-417.

Hattevig G, Kjellman B, Bjorksten B (1993) Appearance of IgE antibodies to ingested and inhaled allergens during the first 12 years of life in atopic and non-atopic children. *Pediatr Allergy Immunol*, **4**, 182-186.

Heinrich J, Gehring U, Douwes J, et al. (2001) Pets and vermin are associated with high endotoxin levels in house dust. *Clin Exp Allergy*, **31**, 1839-1845.

Heinrich J, Hoelscher B, Frye C, et al. (2002) Trends in prevalence of atopic diseases and allergic sensitization in children in Eastern Germany. *Eur Respir J*, **19**, 1040-1046.

Heinrich J, Holscher B, Douwes J, et al. (2003) Reproducibility of allergen, endotoxin and fungi measurements in the indoor environment. *J Expo Anal Environ Epidemiol*, **13**, 152-160.

Heinrich J, Popescu MA, Wjst M, et al. (1998) Atopy in children and parental social class. *Am J Public Health*, **88**, 1319-1324.

Heinrich J and Wichmann HE (2004) Traffic related pollutants in Europe and their effect on allergic disease. *Curr Opin Allergy Clin Immunol*, **4**, 341-348.

Holt PG and Jones CA (2000) The development of the immune system during pregnancy and early life. *Allergy*, **55**, 688-697.

Hosmer D.W. and Lemeshow S (2000) *Applied logistic Regression*, 2 ed. New York, USA, John Wiley and Sons.

Huggins KG and Brostoff J (1975) Local production of specific IgE antibodies in allergic-rhinitis patients with negative skin tests. *Lancet*, **2**, 148-50.

Hyvarinen A, Roponen M, Tiittanen P, et al. (2006) Dust sampling methods for endotoxin - an essential, but underestimated issue. *Indoor Air*, **16**, 20-27.

Illi S, von Mutius E, Lau S, et al. (2006) Perennial allergen sensitisation early in life and chronic asthma in children: a birth cohort study. *Lancet*, **368**, 763-770.

Ingram JM, Sporik R, Rose G, et al. (1995) Quantitative assessment of exposure to dog (Can f 1) and cat (Fel d 1) allergens: relation to sensitization and asthma among children living in Los Alamos, New Mexico. *J Allergy Clin Immunol*, **96**, 449-456.

Instanes C, Hetland G, Berntsen S, et al. (2005) Allergens and endotoxin in settled dust from day-care centers and schools in Oslo, Norway. *Indoor Air*, **15**, 356-362.

Instanes C, Ormstad H, Rydjord B, et al. (2004) Mould extracts increase the allergic response to ovalbumin in mice. *Clin Exp Allergy*, **34**, 1634-1641.

Iossifova YY, Reponen T, Bernstein DI, et al. (2007) House dust (1-3)-beta-d-glucan and wheezing in infants. *Allergy*, **62**, 504-513.

Jacobs DE, Wilson J, Dixon SL, et al. (2009) The relationship of housing and population health: a 30-year retrospective analysis. *Environ Health Perspect*, **117**, 597-604.

Johansson SG, Bieber T, Dahl R, et al. (2004) Revised nomenclature for allergy for global use: Report of the Nomenclature Review Committee of the World Allergy Organization, October 2003. *J Allergy Clin Immunol*, **113**, 832-836.

Karlsson AS and Renstrom A (2005) Human hair is a potential source of cat allergen contamination of ambient air. *Allergy*, **60**, 961-964.

Keil T, Kulig M, Simpson A, et al. (2006) European birth cohort studies on asthma and atopic diseases: I. Comparison of study designs -- a GALEN initiative. *Allergy*, **61**, 221-228.

Kerkhof M, Wijga AH, Brunekreef B, et al. (2009) Effects of pets on asthma development up to 8 years of age: the PIAMA study. *Allergy*, **64**, 1202-1208.

Kitch BT, Chew G, Burge HA, et al. (2000) Socioeconomic predictors of high allergen levels in homes in the greater Boston area. *Environ Health Perspect*, **108**, 301-307.

Kjellman NI (1977) Atopic disease in seven-year-old children. Incidence in relation to family history. *Acta Paediatr Scand*, **66**, 465-471.

Klepeis NE, Nelson WC, Ott WR, et al. (2001) The National Human Activity Pattern Survey (NHAPS): a resource for assessing exposure to environmental pollutants. *J Expo Anal Environ Epidemiol*, **11**, 231-252.

Korppi M, Hyvarinen M, Kotaniemi-Syrjanen A, et al. (2008) Early exposure and sensitization to cat and dog: different effects on asthma risk after wheezing in infancy. *Pediatr Allergy Immunol*, **19**, 696-701.

Kotaniemi-Syrjanen A, Reijonen TM, Romppanen J, et al. (2003) Allergen-specific immunoglobulin E antibodies in wheezing infants: the risk for asthma in later childhood. *Pediatrics*, **111**, e255-e261.

Kulig M, Bergmann R, Edenharter G, et al. (2000) Does allergy in parents depend on allergy in their children? Recall bias in parental questioning of atopic diseases. Multicenter Allergy Study Group. *J Allergy Clin Immunol*, **105**, 274-278.

Lack G (2001) Pediatric allergic rhinitis and comorbid disorders. *J Allergy Clin Immunol*, **108**, S9-15.

Lakind JS and Naiman DQ (2008) Bisphenol A (BPA) daily intakes in the United States: estimates from the 2003-2004 NHANES urinary BPA data. *J Expo Sci Environ Epidemiol*, **18**, 608-615.

Landis JR and Koch GG (1977) The measurement of observer agreement for categorical data. *Biometrics*, **33**, 159-174.

Langley SJ, Goldthorpe S, Craven M, et al. (2005) Relationship between exposure to domestic allergens and bronchial hyperresponsiveness in non-sensitised, atopic asthmatic subjects. *Thorax*, **60**, 17-21.

Lannero E, Kull I, Wickman M, et al. (2002) Environmental risk factors for allergy and socioeconomic status in a birth cohort (BAMSE). *Pediatr Allergy Immunol*, **13**, 182-187.

Lau S, Illi S, Platts-Mills TA, et al. (2005) Longitudinal study on the relationship between cat allergen and endotoxin exposure, sensitization, cat-specific IgG and development of asthma in childhood--report of the German Multicentre Allergy Study (MAS 90). *Allergy*, **60**, 766-773.

Lau S, Illi S, Sommerfeld C, et al. (2000) Early exposure to house-dust mite and cat allergens and development of childhood asthma: a cohort study. Multicentre Allergy Study Group. *Lancet*, **356**, 1392-1397.

Leynaert B, Neukirch C, Kony S, et al. (2004) Association between asthma and rhinitis according to atopic sensitization in a population-based study. *J Allergy Clin Immunol*, **113**, 86-93.

Liccardi G, Cazzola M, Russo M, et al. (2001) Mechanisms and characteristics of airway sensitization to indoor allergens. *Monaldi Arch Chest Dis*, **56**, 55-63.

Lindfors A, Hage-Hamsten M, Rietz H, et al. (1999) Influence of interaction of environmental risk factors and sensitization in young asthmatic children. *J Allergy Clin Immunol*, **104**, 755-762.

Liu AH (2004) Something old, something new: indoor endotoxin, allergens and asthma. *Paediatr Respir Rev*, **5 Suppl A**, 65-71.

Lodrup Carlsen KC, Carlsen KH, Buchmann MS, et al. (2002) Cockroach sensitivity in Norway: a previously unidentified problem? *Allergy*, **57**, 529-533.

Lodrup Carlsen KC, Haland G, Devulapalli CS, et al. (2006a) Asthma in every fifth child in Oslo, Norway: a 10-year follow up of a birth cohort study. *Allergy*, **61**, 454-460.

Lodrup Carlsen KC, Lovik M, Granum B, et al. (2006b) Soluble CD14 at 2 yr of age: gender-related effects of tobacco smoke exposure, recurrent infections and atopic diseases. *Pediatr Allergy Immunol*, **17**, 304-312.

Lodrup Carlsen KC, Magnus P, Carlsen KH (1994) Lung function by tidal breathing in awake healthy newborn infants. *Eur Respir J*, **7**, 1660-1668.

Los H, Postmus PE, Boomsma DI (2001) Asthma genetics and intermediate phenotypes: a review from twin studies. *Twin Res*, **4**, 81-93.

Lowe AJ, Carlin JB, Bennett CM, et al. (2008) Do boys do the atopic march while girls dawdle? *J Allergy Clin Immunol*, **121**, 1190-1195.

Lowe LA, Woodcock A, Murray CS, et al. (2004) Lung function at age 3 years: effect of pet ownership and exposure to indoor allergens. *Arch Pediatr Adolesc Med*, **158**, 996-1001.

Luczynska CM, Li Y, Chapman MD, et al. (1990) Airborne concentrations and particle size distribution of allergen derived from domestic cats (Felis domesticus). Measurements using cascade impactor, liquid impinger, and a two-site monoclonal antibody assay for Fel d I. *Am Rev Respir Dis*, **141**, 361-367.

Malandain H (2004) IgE antibody in the serum--the main problem is cross-reactivity. *Allergy*, **59**, 229.

Mandhane PJ, Sears MR, Poulton R, et al. (2009) Cats and dogs and the risk of atopy in childhood and adulthood. *J Allergy Clin Immunol*, **124**, 745-750.

Mantyjarvi R, Rautiainen J, Virtanen T (2000) Lipocalins as allergens. *Biochim Biophys Acta*, **1482**, 308-317.

Mapp CE, Boschetto P, Maestrelli P, et al. (2005) Occupational asthma. *Am J Respir Crit Care Med*, **172**, 280-305.

Marti A, Moreno-Aliaga MJ, Hebebrand J, et al. (2004) Genes, lifestyles and obesity. *Int J Obes Relat Metab Disord*, **28 Suppl 3**, 29-36.

Martinez FD (2009) The origins of asthma and chronic obstructive pulmonary disease in early life. *Proc Am Thorac Soc*, **6**, 272-277.

Masoli M, Fabian D, Holt S, et al. (2004) The global burden of asthma: executive summary of the GINA Dissemination Committee report. *Allergy*, **59**, 469-478.

Michel O, Nagy AM, Schroeven M, et al. (1997) Dose-response relationship to inhaled endotoxin in normal subjects. *Am J Respir Crit Care Med*, **156**, 1157-1164.

Miossec P, Korn T, Kuchroo VK (2009) Interleukin-17 and type 17 helper T cells. *N Engl J Med*, **361**, 888-898.

Murphy K, Travers O, Walport M (2008) *Janeway's Immunobiology*, 7 ed. New York, Garland Science, Taylor & Francis Group.

Murray AB, Ferguson AC, Morrison BJ (1983) The frequency and severity of cat allergy vs. dog allergy in atopic children. *J Allergy Clin Immunol*, **72**, 145-149.

Nafstad P, Jaakkola JJ, Hagen JA, et al. (1996) Breastfeeding, maternal smoking and lower respiratory tract infections. *Eur Respir J*, **9**, 2623-2629.

Nafstad P, Magnus P, Gaarder PI, et al. (2001) Exposure to pets and atopy-related diseases in the first 4 years of life. *Allergy*, **56**, 307-312.

Nafstad P, Oie L, Mehl R, et al. (1998) Residential dampness problems and symptoms and signs of bronchial obstruction in young Norwegian children. *Am J Respir Crit Care Med*, **157**, 410-414.

Nicholas C, Wegienka G, Havstad S, et al. (2009) How accurately do young adults recall childhood pets? A validation study. *Am J Epidemiol*, **170**, 388-392.

Nielsen G.D., Larsen, S. T., Hansen, J. S. et al. (2005) Adjuvant effect of MEHP and DEHP by inhalation in a murine model. Interim results. *Invited presentation at 31st Annual Summer Metting, The Toxocology forum*, Aspen, Colorado.

Novak M and Vetvicka V (2008) Beta-glucans, history, and the present: immunomodulatory aspects and mechanisms of action. *J Immunotoxicol*, **5**, 47-57.

O'Connor G, Sparrow D, Taylor D, et al. (1987) Analysis of dose-response curves to methacholine. An approach suitable for population studies. *Am Rev Respir Dis*, **136**, 1412-1417.

Oboki K, Ohno T, Saito H, et al. (2008) Th17 and allergy. *Allergol Int*, **57**, 121-134.

Omenaas E, Bakke P, Elsayed S, et al. (1994) Total and specific serum IgE levels in adults: relationship to sex, age and environmental factors. *Clin Exp Allergy*, **24**, 530-539.

Ormstad H, Groeng EC, Lovik M, et al. (2000) The fungal cell wall component beta-1,3-glucan has an adjuvant effect on the allergic response to ovalbumin in mice. *J Toxicol Environ Health A*, **61**, 55-67.

Ormstad H, Johansen BV, Gaarder PI (1998) Airborne house dust particles and diesel exhaust particles as allergen carriers. *Clin Exp Allergy*, **28**, 702-708.

Ormstad H, Namork E, Gaarder PI, et al. (1995) Scanning electron microscopy of immunogold labeled cat allergens (Fel d 1) on the surface of airborne house dust particles. *J Immunol Methods*, **187**, 245-251.

Owen CE (2007) Immunoglobulin E: role in asthma and allergic disease: lessons from the clinic. *Pharmacol Ther*, **113**, 121-133.

Ozdemir C, Akdis M, Akdis CA (2009) T regulatory cells and their counterparts: masters of immune regulation. *Clin Exp Allergy*, **39**, 626-639.

Pallasaho P, Meren M, Raukas-Kivioja A, et al. (2005) Different labelling of obstructive airway diseases in Estonia, Finland, and Sweden. *Eur J Epidemiol*, **20**, 975-983.

Park JH, Gold DR, Spiegelman DL, et al. (2001) House dust endotoxin and wheeze in the first year of life. *Am J Respir Crit Care Med*, **163**, 322-328.

Passalacqua G and Ciprandi G (2008) Allergy and the lung. *Clin Exp Immunol*, **153 Suppl 1:12-6.**, 12-16.

Pearce N, Douwes J, Beasley R (2000) Is allergen exposure the major primary cause of asthma? *Thorax*, **55**, 424-431.

Perzanowski MS, Miller RL, Thorne PS, et al. (2006) Endotoxin in inner-city homes: associations with wheeze and eczema in early childhood. *J Allergy Clin Immunol*, **117**, 1082-1089.

Perzanowski MS, Ronmark E, Nold B, et al. (1999) Relevance of allergens from cats and dogs to asthma in the northernmost province of Sweden: schools as a major site of exposure. *J Allergy Clin Immunol*, **103**, 1018-1024.

Perzanowski MS, Ronmark E, Platts-Mills TA, et al. (2002) Effect of cat and dog ownership on sensitization and development of asthma among preteenage children. *Am J Respir Crit Care Med*, **166**, 696-702.

Phipatanakul W (2005) Allergic rhinoconjunctivitis: epidemiology. *Immunol Allergy Clin North Am*, **25**, 263-81, vi.

Platts-Mills T, Vaughan J, Squillace S, et al. (2001) Sensitisation, asthma, and a modified Th2 response in children exposed to cat allergen: a population-based cross-sectional study. *Lancet*, **357**, 752-756.

Platts-Mills TA, Woodfolk JA, Erwin EA, et al. (2004) Mechanisms of tolerance to inhalant allergens: the relevance of a modified Th2 response to allergens from domestic animals. *Springer Semin Immunopathol*, **25**, 271-279.

Ponsonby AL, Glasgow N, Pezic A, et al. (2008) A temporal decline in asthma but not eczema prevalence from 2000 to 2005 at school entry in the Australian Capital Territory with further consideration of country of birth. *Int J Epidemiol*, **37**, 559-569.

Postma DS (2007) Gender differences in asthma development and progression. *Gend Med*, **4 Suppl B**, 133-146.

Poulsen LK (2005) In search of a new paradigm: mechanisms of sensitization and elicitation of food allergy. *Allergy*, **60**, 549-558.

Quanjer PH, Tammeling GJ, Cotes JE, et al. (1993) Lung volumes and forced ventilatory flows. Report Working Party Standardization of Lung Function Tests, European Community for Steel and Coal. Official Statement of the European Respiratory Society. *Eur Respir J Suppl*, **16**, 5-40.

Reininger R, Varga EM, Zach M, et al. (2007) Detection of an allergen in dog dander that cross-reacts with the major cat allergen, Fel d 1. *Clin Exp Allergy*, **37**, 116-124.

Ricci G, Righetti F, Menna G, et al. (2005) Relationship between Bet v 1 and Bet v 2 specific IgE and food allergy in children with grass pollen respiratory allergy. *Mol Immunol*, **42**, 1251-1257.

Richardson G, Eick S, Jones R (2005) How is the indoor environment related to asthma?: literature review. *J Adv Nurs*, **52**, 328-339.

Ring J, Kramer U, Schafer T, et al. (1999) Environmental risk factors for respiratory and skin atopy: results from epidemiological studies in former East and West Germany. *Int Arch Allergy Immunol*, **118**, 403-407.

Ronchetti R, Bonci E, Cutrera R, et al. (1992) Enhanced allergic sensitisation related to parental smoking. *Arch Dis Child*, **67**, 496-500.

Ronchetti R, Rennerova Z, Barreto M, et al. (2007) The prevalence of atopy in asthmatic children correlates strictly with the prevalence of atopy among nonasthmatic children. *Int Arch Allergy Immunol*, **142**, 79-85.

Ronmark E, Bjerg A, Perzanowski M, et al. (2009) Major increase in allergic sensitization in schoolchildren from 1996 to 2006 in northern Sweden. *J Allergy Clin Immunol*, **124**, 357-63, 63.

Rosenstreich DL, Eggleston P, Kattan M, et al. (1997) The role of cockroach allergy and exposure to cockroach allergen in causing morbidity among inner-city children with asthma. *N Engl J Med*, **336**, 1356-1363.

Rylander R, Bake B, Fischer JJ, et al. (1989) Pulmonary function and symptoms after inhalation of endotoxin. *Am Rev Respir Dis*, **140**, 981-986.

Rylander R, Norrhall M, Engdahl U, et al. (1998) Airways inflammation, atopy, and (1 -> 3)-beta-D-glucan exposures in two schools. *Am J Respir Crit Care Med*, **158**, 1685-1687.

Samuelsen M, Nygaard UC, Lovik M (2008) Allergy adjuvant effect of particles from wood smoke and road traffic. *Toxicology*, **246**, 124-131.

Sandin A, Bjorksten B, Braback L (2004) Development of atopy and wheezing symptoms in relation to heredity and early pet keeping in a Swedish birth cohort. *Pediatr Allergy Immunol*, **15**, 316-322.

Sanz ML, Prieto I, Garcia BE, et al. (1996) Diagnostic reliability considerations of specific IgE determination. *J Investig Allergol Clin Immunol*, **6**, 152-161.

Sauerbrei W and Royston P (2009) Building multivariable prognostic and diagnostic models: transformation of the predictors by using fractional polynomials. *J Roy Stat Soc Ser A*, **162**, 71-94.

Schram D, Doekes G, Boeve M, et al. (2005) Bacterial and fungal components in house dust of farm children, Rudolf Steiner school children and reference children--the PARSIFAL Study. *Allergy*, **60**, 611-618.

Schram-Bijkerk D, Doekes G, Boeve M, et al. (2006) Exposure to microbial components and allergens in population studies: a comparison of two house dust collection methods applied by participants and fieldworkers. *Indoor Air*, **16**, 414-425.

Schram-Bijkerk D, Doekes G, Douwes J, et al. (2005) Bacterial and fungal agents in house dust and wheeze in children: the PARSIFAL study. *Clin Exp Allergy*, **35**, 1272-1278.

Schwartz DA, Donham KJ, Olenchock SA, et al. (1995) Determinants of longitudinal changes in spirometric function among swine confinement operators and farmers. *Am J Respir Crit Care Med*, **151**, 47-53.

Sicherer SH and Sampson HA (2006) 9. Food allergy. *J Allergy Clin Immunol*, **117**, S470-S475.

Siersted HC, Boldsen J, Hansen HS, et al. (1998) Population based study of risk factors for underdiagnosis of asthma in adolescence: Odense schoolchild study. *BMJ*, **316**, 651-655.

Simpson A and Custovic A (2005) Pets and the development of allergic sensitization. *Curr Allergy Asthma Rep*, **5**, 212-220.

Skorge TD, Eagan TM, Eide GE, et al. (2005) Indoor exposures and respiratory symptoms in a Norwegian community sample. *Thorax*, **60**, 937-942.

Sly PD, Boner AL, Bjorksten B, et al. (2008) Early identification of atopy in the prediction of persistent asthma in children. *Lancet*, **372**, 1100-1106.

Spergel JM and Paller AS (2003) Atopic dermatitis and the atopic march. *J Allergy Clin Immunol*, **112**, S118-S127.

Sporik R, Holgate ST, Platts-Mills TA, et al. (1990) Exposure to house-dust mite allergen (Der p I) and the development of asthma in childhood. A prospective study. *N Engl J Med*, **323**, 502-507.

Sporik R, Ingram JM, Price W, et al. (1995) Association of asthma with serum IgE and skin test reactivity to allergens among children living at high altitude. Tickling the dragon's breath. *Am J Respir Crit Care Med*, **151**, 1388-1392.

Stanojevic S, Wade A, Cole TJ, et al. (2009) Spirometry centile charts for young Caucasian children: the Asthma UK Collaborative Initiative. *Am J Respir Crit Care Med*, **180**, 547-552.

Strachan DP (2000) Family size, infection and atopy: the first decade of the "hygiene hypothesis". *Thorax*, **55 Suppl 1**, 2-10.

Svanes C, Dharmage S, Sunyer J, et al. (2008) Long-term reliability in reporting of childhood pets by adults interviewed twice, 9 years apart. Results from the European Community Respiratory Health Survey I and II. *Indoor Air*, **18**, 84-92.

Svanes C, Heinrich J, Jarvis D, et al. (2003) Pet-keeping in childhood and adult asthma and hay fever: European community respiratory health survey. *J Allergy Clin Immunol*, **112**, 289-300.

Svanes C, Jarvis D, Chinn S, et al. (1999) Childhood environment and adult atopy: results from the European Community Respiratory Health Survey. *J Allergy Clin Immunol*, **103**, 415-420.

Svanes C, Zock JP, Anto J, et al. (2006) Do asthma and allergy influence subsequent pet keeping? An analysis of childhood and adulthood. *J Allergy Clin Immunol*, **118**, 691-698.

Svartengren M, Falk R, Linnman L, et al. (1987) Deposition of large particles in human lung. *Exp Lung Res*, **12**, 75-88.

The International Study of Asthma and Allergies in Childhood (ISAAC) Steering Committee (1998) Worldwide variations in the prevalence of asthma symptoms: the International Study of Asthma and Allergies in Childhood (ISAAC). *Eur Respir J*, **12**, 315-335.

Thorne PS, Duchaine C, Douwes J, et al. (2004) Working group report 4: Exposure assessment for biological agents. *Am J Ind Med*, **46**, 419-422.

Tian X, Takamoto M, Sugane K (2003) Bisphenol A promotes IL-4 production by Th2 cells. *Int Arch Allergy Immunol*, **132**, 240-247.

Torrent M, Sunyer J, Garcia R, et al. (2007) Early-life allergen exposure and atopy, asthma, and wheeze up to 6 years of age. *Am J Respir Crit Care Med*, **176**, 446-453.

Torrent M, Sunyer J, Munoz L, et al. (2006) Early-life domestic aeroallergen exposure and IgE sensitization at age 4 years. *J Allergy Clin Immunol*, **118**, 742-748.

Tovey ER (2008) Allergen avoidance. *Curr Allergy Asthma Rep*, **8**, 126-132.

Tovey ER, Almqvist C, Li Q, et al. (2008) Nonlinear relationship of mite allergen exposure to mite sensitization and asthma in a birth cohort. *J Allergy Clin Immunol*, **122**, 114-118.

Tschopp JM, Sistek D, Schindler C, et al. (1998) Current allergic asthma and rhinitis: diagnostic efficiency of three commonly used atopic markers (IgE, skin prick tests, and Phadiatop). Results from 8329 randomized adults from the SAPALDIA Study. Swiss Study on Air Pollution and Lung Diseases in Adults. *Allergy*, **53**, 608-613.

Vassallo R and Limper AH (1999) Fungal beta-glucan can yield false-positive results with the limulus amebocyte lysate endotoxin assay. *Chest*, **116**, 583-584.

Virtanen T (2001) Lipocalin allergens. *Allergy*, **56 Suppl 67,** 48-51.

Volmer T (2001) The socio-economics of asthma. *Pulm Pharmacol Ther*, **14**, 55-60.

Von Hertzen LC and Haahtela T (2004) Asthma and atopy - the price of affluence? *Allergy*, **59**, 124-137.

von Mutius E (2000) The environmental predictors of allergic disease. *J Allergy Clin Immunol*, **105**, 9-19.

von Mutius E, Braun-Fahrlander C, Schierl R, et al. (2000) Exposure to endotoxin or other bacterial components might protect against the development of atopy. *Clin Exp Allergy*, **30**, 1230-1234.

von Mutius E, Weiland SK, Fritzsch C, et al. (1998) Increasing prevalence of hay fever and atopy among children in Leipzig, East Germany. *Lancet*, **351**, 862-866.

Wahn U, Lau S, Bergmann R, et al. (1997) Indoor allergen exposure is a risk factor for sensitization during the first three years of life. *J Allergy Clin Immunol*, **99**, 763-769.

Wan H, Winton HL, Soeller C, et al. (1999) Der p 1 facilitates transepithelial allergen delivery by disruption of tight junctions. *J Clin Invest*, **104**, 123-133.

Warner AM, Bjorksten B, Munir AK, et al. (1996) Childhood asthma and exposure to indoor allergens: low mite levels are associated with sensitivity. *Pediatr Allergy Immunol*, **7**, 61-67.

Weinmayr G, Forastiere F, Weiland SK, et al. (2008) International variation in prevalence of rhinitis and its relationship with sensitisation to perennial and seasonal allergens. *Eur Respir J*, **32**, 1250-1261.

Wickens K, Lane J, Siebers R, et al. (2004) Comparison of two dust collection methods for reservoir indoor allergens and endotoxin on carpets and mattresses. *Indoor Air*, **14**, 217-222.

Wickman M, Lilja G, Soderstrom L, et al. (2005) Quantitative analysis of IgE antibodies to food and inhalant allergens in 4-year-old children reflects their likelihood of allergic disease. *Allergy*, **60**, 650-657.

Wickman M, Nordvall SL, Pershagen G, et al. (1993) Sensitization to domestic mites in a cold temperate region. *Am Rev Respir Dis*, **148**, 58-62.

Williams HC, Strachan DP, Hay RJ (1994) Childhood eczema: disease of the advantaged? *BMJ*, **308**, 1132-1135.

Williams LK, Ownby DR, Maliarik MJ, et al. (2005) The role of endotoxin and its receptors in allergic disease. *Ann Allergy Asthma Immunol*, **94**, 323-332.

Wittassek M, Heger W, Koch HM, et al. (2007) Daily intake of di(2-ethylhexyl)phthalate (DEHP) by German children -- A comparison of two estimation models based on urinary DEHP metabolite levels. *Int J Hyg Environ Health*, **210**, 35-42.

Wong GW, Hui DS, Chan HH, et al. (2001) Prevalence of respiratory and atopic disorders in Chinese schoolchildren. *Clin Exp Allergy*, **31**, 1225-1231.

Woodcock A and Custovic A (1998) ABC of allergies. Avoiding exposure to indoor allergens. *BMJ*, **316**, 1075-1078.

Wuthrich B (1999) Clinical aspects, epidemiology, and prognosis of atopic dermatitis. *Ann Allergy Asthma Immunol*, **83**, 464-470.

Yeatts K, Davis KJ, Sotir M, et al. (2003) Who gets diagnosed with asthma? Frequent wheeze among adolescents with and without a diagnosis of asthma. *Pediatrics*, **111**, 1046-1054.

Zhang GH, Baek L, Buchardt O, et al. (1994) Differential Blocking of Coagulation-Activating Pathways of Limulus Amebocyte Lysate. *J Clin Microbiol*, **32**, 1537-1541.

Zureik M, Neukirch C, Leynaert B, et al. (2002) Sensitisation to airborne moulds and severity of asthma: cross sectional study from European Community respiratory health survey. *BMJ*, **325**, 411-414.

I

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Supporting information for Paper I

Table S1 Information from questionnaires at birth to age 2 and from interview at age 10 (translated from Norwegian)

Index child's age	**Questions**	**Outcomes/definitions**
6, 12, 18 and 24 months	Has the child had atopic eczema during the last 6 months?	**Atopic eczema by age 2**
10 years	Has the child ever had any of the following symptoms irrespective of a cold: runny nose, blocked nose, sneezing and/or itching/runny eyes?	**Allergic rhinoconjunctivitis ever**
	Has the child ever had any allergies confirmed by a doctor? (not including food-allergies)	
	Has the child ever had a diagnosis of asthma confirmed by a doctor?	**Ever diagnosed with asthma**
	Has the child previously experienced respiratory (wheezing) symptoms caused by furry pets?	**Wheezing symptoms due to furry pets**
Birth	Have any of the following family members ever had asthma: Father, mother or siblings?	**Siblings or parents with a history of asthma**
	Have any of the following family members ever had rhinoconjunctivitis: Father, mother or siblings?	**Siblings or parents with a history of rhinoconjunctivitis**
	What was the total family gross income last year? 1:<100, 2:100-199, 3:200-299, 4:300-500,5:>500 in 1000 NOK	**Family income**
Birth	Do mother and/or father smoke at home (sometimes or daily)?	**Parental smoking at birth**
10 years	Did mother and/or father smoke at home a) before school-age (age 6-7) b) after school-age	**Parental smoking** (after child's birth)
Birth and 10 years	Which type of house does the child live in? a) flat b) detached/semi-detached house.	**Type of house at birth** **Moved from flat to house** (same type of housing vs moved from flat to house)
Birth	Father and mother highest completed education: a) compulsory (± 1-2 year county college) b) college c) university	**Parents' education** (highest education by either parent)
10 years	With whom does the child live with more than >50% of the time? a) mother and father b) mother c) father	**Single parenthood**
	Have the family avoided pets or removed pets due to allergy in the family and/or to prevent the child from developing allergies?	**Reported pet avoidance**
Birth, 6,12,18,24 months and 10 years	Do you keep furry pets at home? If yes: a) cat b) dog c) other types of furry pets (rodents)	**Cats, dogs or other furry pets at birth** **Change in pet ownership from birth to age 10**
10 years	Did you keep cats, dogs or other furry pets at home during the child's first year of life?	Recall information regarding pet keeping first year of life

Table S2 Predictor variables associated with reported pet avoidance

Predictor variables	% with reported pet avoidance	Adjusted OR (95% CI)[a]
Index child with atopic eczema ever during the 2 first years of life		
No	24.4	1
Yes	43.2	1.88(1.39-2.54)
Index child with allergic rhinoconjunctivitis ever		
No	27.8	1
Yes	61.8	3.05(1.99-4.67)
Index child ever diagnosed with asthma		
No	26.9	1
Yes	48.2	1.83(1.31-2.58)
Parental rhinoconjunctivitis[b]		
No	25.2	1
Yes	48.7	2.36(1.72-3.23)
Parental asthma[b]		
No	28.5	1
Yes	57.0	2.41(1.59-3.65)
Parents' education		
Compulsory	18.6	1
College	31.5	2.29(0.96-5.48)
University	34.5	2.86(1.21-6.78)

[a] Adjusted odds ratios (OR) and 95% confidence intervals (CI) estimated by bivariate and multivariate logistic regression models (backward (manual) elimination technique). The other variables initially included, but not significant in the final model, were index child's recurrent bronchial obstruction (rBO) by age 2 years, siblings asthma or rhinoconjunctivitis and parental smoking ever.

[b] reported at index child's birth

II

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Supporting information for Paper II

Methods

Dust sampling and extractions

The dust samples were collected by the parents according to detailed written instructions. They were asked to use new vacuum cleaner bags, vacuum the floor of the living area of the house and collect the entire sample of dust from the bags. The dust samples were sent in plastic bags to the Norwegian Institute of Public Health and stored at -20°C until extraction and analysis. Endotoxin, allergens and ß(1,3)-glucans were extracted sequentially. First, 5 to 40 ml 0.05% (v/v) Tween-20 in pyrogen-free water was added, determined by the net dust weight (<0.5 g, 5 ml; 0.5-1.0 g, 10 ml; 1.0 to 2.0 g, 20 ml; >2.0g, 40 ml). The suspensions were incubated in an end-over-end roller for 1 h at room temperature, and after centrifugation (15 min, 1000 x g) the upper 10% of the supernatant was harvested and stored in four aliquots at -20°C for endotoxin analysis. For the second step, the removed supernatant was replaced with the same volume of 10 x concentrated phosphate-buffered saline (PBS), thus changing the extraction medium into PBS-0.045% Tween-20 (PBT). After re-suspension and thorough mixing of the first pellet in the new medium, incubation was continued in the end-over-end roller (1 h), followed by centrifugation (15 min, 2000 x g). The supernatant was harvested and stored at -20°C for analysis of cat, dog and mite allergens. The remaining dust pellets were re-suspended in the original volume of PBT (0.05%), incubated in an end-over-end roller again for 15 min, autoclaved for 1 h at 120°C and incubated in an end-over-end roller again for 15 min. After centrifugation (15 min, 1000 x g), the supernatant was harvested and stored at -20°C until analysis for ß(1,3)-glucans.

Analyses of cat, dog and mite allergens, endotoxin and ß(1,3)-glucans

The concentrations of cat allergen (*Felis domesticus* Fel d1) and mite allergen (*Dermatophagoides pteronyssinus* Der p1) were determined by enzyme-linked

immunosorbent assay following mainly the manufacturer's instructions (Indoor Biotechnologies, Clewyd, UK) [1]. Monoclonal antibodies were used as capture and detection antibodies. The enzyme-labelled secondary detection reagent used in the assays was alkaline phosphatase-conjugated avidin (DAKO A/S, Glostrup, Denmark). A series of dilutions of reference cat dander preparation or Der p1 extract (both Indoor Biotechnologies) were included on every plate to provide a standard curve. The concentrations of dog allergen (*Canis familiaris* Can f1) was measured with a monoclonal capture antibody (Indoor Biotechnologies), polyclonal rabbit anti-Can f1 as detection antibody (ALK laboratories, Hørsholm, Denmark) and alkaline phosphatase-labeled donkey anti-rabbit IgG (Jackson ImmunoResearch Laboratories, West Grove, USA) as secondary enzyme-labelled antibody. A series of dilutions of reference dog hair and dander extract (NIBSC, Hertfordshire, UK) was included on every plate to provide a standard curve.

ß(1,3)-glucans were measured with an inhibition Enzyme Immuno Assay (EIA) [2]. Flat-bottom microtiter plates were incubated overnight at 4°C with Laminarin (BioChemika (Sigma-Aldrich, St.Louis, USA)) (16 µg/ml) in PBS (200 µl per well). After washing, 300 µl of 0.2% gelatine in PBT (PBTG) was applied, the mixture was incubated at 37°C for 1 h, and the gelatine solution was discarded. A test sample or a laminarin standard (100 µl diluted in PBTG) was then added to a microwell and subsequently mixed with an equal volume of affinity-purified anti- ß(1,3)-glucan antibodies (Batch 9819, provided by Dr. G. Doekes, IRAS, Utrecht, The Netherlands) diluted 1/40 000 in PBTG. The microtiter plate was then shaken at 37°C for 1.5 h. After washing, 200 µl of peroxidase-labelled swine anti-rabbit immunoglobulin antibodies (DAKO A/S) diluted 1/2000 in PBTG was added and the mixture was shaken for 1 h at 37°C. After washing, 200 µl of SIGMA *FAST*^{TM} OPD (o-phenylenediamine dihydrochloride) was added and the mixture was incubated for 30 min at

20°C. The enzyme reaction was terminated by addition of 50 µl of 2 M HCl, and the optical density was read at 490 nm. Concentration was expressed as µg ß(1,3)-glucan per gram dust.

References

1. Chapman MD, Heymann PW, Wilkins SR, Brown MJ, Platts-Mills TA. Monoclonal immunoassays for major dust mite (Dermatophagoides) allergens, Der p I and Der f I, and quantitative analysis of the allergen content of mite and house dust extracts. J Allergy Clin Immunol 1987; 80:184-94.

2. Douwes J, Doekes G, Montijn R, Heederik D, Brunekreef B. Measurement of beta(1-->3)-glucans in occupational and home environments with an inhibition enzyme immunoassay. Appl Environ Microbiol 1996; 62:3176-82.

III

RHINITIS IN CHILDREN: CO-MORBIDITIES AND PHENOTYPES

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ABSTRACT

Rhinitis and its co-morbidities affect quality of life, and in children little is known. We therefore aimed to describe the co-morbidities and phenotypes of current rhinitis in 10-year-old Norwegian children. Information on rhinitis, asthma, allergic sensitization and lung function tests was retrieved from clinical examinations and structured parental interviews of 1019 children included in the birth cohort study, ECA, in Oslo. Current rhinitis was reported in 254 (25.0%) of the children, and 87% had at least one allergy-related co-morbidity of rhinitis, with conjunctivitis the most prevalent (75.6%). Overall, 11.8% of the children had conjunctivitis combined with current asthma and atopic eczema. Children with rhinitis were also more likely to have severe bronchial hyperresponsiveness (BHR) ($PD_{20} \leq 1$ µmol methacholine) than children without rhinitis (17.5% and 5.8%, respectively, $p<0.001$), as well as BHR ($PD_{20} \leq 8$ µmol). The children with rhinitis *and* allergic sensitization (72.8%) had more frequently BHR, severe BHR and conjunctivitis, but did not differ with respect to any other co-morbidity from children with rhinitis *without* allergic sensitization. Children with symptoms reported both by exposure to pollen and furry pets had more often asthma, wheeze and BHR compared to the children who reported rhinitis symptoms only when exposed to pollen (91.9% of these had symptoms in spring/summer only). In conclusion, current rhinitis is closely associated with conjunctivitis, asthma, atopic eczema, and BHR in childhood. Conjunctivitis and BHR were most strongly linked to rhinitis with allergic sensitization.

Keywords: BHR, children, co-morbidity, medication, phenotypes, rhinitis, triggers of rhinitis

INTRODUCTION

Chronic rhinitis is a common disease, which together with frequent co-morbidities such as asthma, bronchial hyperresponsiveness (BHR) (1, 2), conjunctivitis (3) and otitis media (1, 4) may affect quality of life (1, 2, 5). The co-morbidities have been described by the type of allergens associated with the rhinitis symptoms, and rhinitis related to exposure from perennial allergens has been more closely associated with asthma (2) and BHR (6) than with rhinitis related to pollen exposure. It is, however, unclear whether patients who are sensitized to more than one allergen (polysensitization) have more co-morbidities and more severe phenotypes than those who are monosensitized (7). Although co-morbidities of allergic rhinitis are well described in adult populations, less is known in childhood (1, 7).

IgE-mediated hypersensitivity of the nose often referred to as allergic rhinitis (8), is currently classified into intermittent and persistent, as well as mild or moderate-severe (9). Although allergens are common elicitors of rhinitis symptoms, rhinitis may also be precipitated by infections, occupational exposure, drugs, hormones, foods, tobacco smoke, emotion and physical or chemical triggers (9). For practical purposes and lack of adequate phenotype categories in childhood, the majority of children with rhinitis symptoms outside respiratory infections have been classified by the presence or absence of IgE or skin-test reactivity to allergens, into allergic or non-allergic rhinitis (3). However, the role of allergens in elicitation of rhinitis in the absence of evidence of systemic IgE sensitization may be underappreciated, as local IgE production may underlie the disease expression (10), and little is known on the phenotypes and co-morbidities of rhinitis in children without detectable systemic IgE sensitization (7, 11).

We therefore aimed to investigate the co-morbidities of current rhinitis in 10-year-old children and secondly to describe the clinical phenotypes identified and the treatment reported for rhinitis in this age group.

MATERIAL AND METHODS

Study design and subjects

The present study is part of the birth cohort study The Environment and Childhood Asthma Study (ECA) initiated in Oslo in 1992 and described in detail elsewhere (12). The study enrolled 3754 healthy full-term babies whose parents were sufficiently skilled in Norwegian language to complete the 6-monthly questionnaire from birth to 2 years of age.

Within the birth cohort 802 children, not differing from the entire general birth cohort population with respect to most relevant factors (12), had lung function measured at birth (13). At 2 years of age a nested case-control study recruited 612 children from the entire ECA study, including children with recurrent or persistent bronchial obstruction (rBO) by 2 years and controls with no bronchial obstruction born closest in time to the cases (14). The children were clinically examined 1-2 times between birth and the age of 2, with at least one investigation in 562 children (figure 1).

All the 1215 children with lung function measured at birth and/or a clinical investigation by 2 years were invited to participate in a 10-year follow-up. In total 1019/1215 (84%) (figure 1) of the children participated in the 10-year follow-up study with two clinical examinations performed 2-7 days apart and at least 4 weeks after any suspicion of respiratory tract infection. The examinations included blood tests, skin prick tests for allergic sensitization, lung function by forced flow-volume measurements, bronchial hyperresponsiveness measures by methacholine challenge test and a treadmill exercise test, as well as a parental structured interview.

Comparison of the demographic birth characteristics of the children included in the follow-up and those who were not included have previously been described (15). The included (n=1019) and non-included children (n=2735) were in general comparable, except that the included children had more often parents and sibling with rhinoconjunctivitis and asthma than the non-included children (31.7% and 26.9%, respectively, p=0.004 for rhinoconjunctivitis) and 17.7% *vs* 14.8%, respectively (p=0.03) for asthma.

Written informed consent was obtained from the parents, and the study was approved by the Regional Committee for Ethics in Medical Research and the Norwegian Data Inspectorate and reported to the Norwegian Biobank Registry.

Methods

The 10-year follow-up

A parental structured interview included central ISAAC questions related to airway symptoms of the child, in addition to detailed questions regarding environmental exposure, life-style, diseases (index child and family) and medication. The questions related to rhinitis focused upon symptoms, medication, triggers, and possible impact the symptoms had on daily life activities (table 1).

Skin prick tests (SPT) were performed according to the European standard (16) with the following standardized allergen extracts from ALK (Soluprick®, ALK-Abello, Denmark): House dust mites (HDM) (*Dermatophagoides pteronyssinus* and *Dermatophagoides farinae*), German cockroach, dog, cat and rabbit, birch, timothy (grass) and mugwort pollens, moulds (*Cladosporium herbarium* and *Alternaria*), egg white, milk, peanut and codfish. SPT were considered positive with a mean wheal diameter at least 3 mm larger than the negative control (saline).

Total and specific serum IgE (IgE and sIgE, respectively) were analysed with a radioallergosorbent fluorescence immunoassay (ImmunoCAP® system, Phadia, Uppsala, Sweden) according to the instructions by the manufacturer. Specific IgE was analysed with the same panel of allergens as for SPT and considered positive with sIgE ≥0.35 kU/L. The lower detection limit was 0.1 kU_A/L for sIgE and 0.1 PAU/L for total IgE.

Baseline spirometry was measured according to European standards (17) on a Sensor Medics Vmax 20c (SensorMedics Diagnostics, Yorba Linda, CA, USA) prior to and after methacholine and exercise challenge tests on separate days. Lung function was reported as per cent predicted of forced expiratory flow at 50% of vital capacity (FEF_{50}) and forced expiratory volume in one second (FEV_1) [reference values of healthy 10-year old children from the ECA-study obtained by methods described by Stanojevic et al. (18)].

BHR was tested directly and indirectly, respectively by:

a) Methacholine challenge test assessed according to international guidelines (19), by doubling the cumulative dose of methacholine and reported as the dose of methacholine causing a fall in FEV_1 of 20% from the (post-saline) baseline value (PD_{20} methacholine)
b) A standardized exercise test (20) performed on the second day by a 6-8 minutes treadmill run at 95% estimated maximum pulse level, and an incline of 5% of the treadmill. The response was measured as maximum fall in FEV_1 assessed before and

3, 6, 10, and 15 minutes after running. The exercise test was considered positive for exercise-induced bronchoconstriction (*EIB*) with a fall in FEV_1>10% of baseline FEV_1

Definitions

Allergy-related co-morbidities were defined as current conjunctivitis, asthma, atopic eczema, and otitis media.

Current rhinitis was present in a child reporting at least one of the following symptoms during the last 12 months (without a cold): runny nose, blocked nose, or sneezing (table 1). *Conjuntivitis* required a report of itchy/runny eyes during the last 12 months.

Rhinitis with allergic sensitization was defined in a child with rhinitis combined with at least one positive SPT and/or sIgE to any of the *inhalant* allergens: *seasonal* (pollens or moulds) or *perennial* (house dust mites, cat, dog, rabbit or cockroach). *Mixed sensitization* was defined with SPT/sIgE positive to at least one seasonal *and* one perennial allergen.

BHR was defined as a categorical variable with three groups: PD_{20} methacholine ≤1μmol, >1 to ≤8 μmol and >8 μmol, and as a continuous variable with the methacholine dose-response slope (DRS) calculated from each individual (per cent decline in FEV_1 after the final dose of methacholine divided by the cumulative dose of methacholine inhaled) (21).

Current asthma was defined in children by the presence of at least two of the three criteria; asthma symptoms, doctor diagnosis of asthma and/or use of asthma medication, and either symptoms or use of asthma medication within the last 12 months and/or a positive exercise challenge test at the 10 year follow-up visit (12).

Current wheeze required a positive response to the question "Has your child experienced dyspnoea, chest tightness and/or wheezing during the last 12 months?"

Otitis media was defined by reported otitis media during the last 12 months, and a*topic eczema* by a positive response to present or having ever had atopic eczema.

Statistical analyses

To adjust for the enrichment of children with bronchial obstruction during the first 2 years of life, an overall prevalence of rhinitis was calculated by the weighted mean of the prevalence of rhinitis for the following groups: (i) rBO by age 2 (ii) controls, and (iii) the follow-up children who were not included in the case-control group. The rhinitis prevalence was calculated for girls and boys separately (six groups in total), and the weight was the number of children in each group. The calculated weighted prevalence is reported with confidence intervals. Categorical variables were compared using Pearson's chi-square test. One-way

ANOVA analysis was used to compare DRS and total IgE (both log-transformed) and presented as geometric mean with 95% confidence intervals (95% CI). FEV_1 and FEF_{50} % predicted was presented as mean with 95% CI. A logistic regression analysis was used to assess the association between current rhinitis and increasing number of sensitizing allergens. P-values ≤0.05 were considered significant. Statistical analyses were performed with Statistical Package for Social Sciences (SPSS version 14.0, SPSS Inc., Chicago, Illinois, USA).

RESULTS

Prevalence of symptoms of current rhinitis

Information on current rhinitis was available for 1015/1019 children from the 10-year follow-up, and 254 (25.0%) of these children had symptoms of current rhinitis. More than one symptom was reported by 72%. The crude prevalence was similar to the calculated prevalence adjusting for the enrichment of children with rBO during the first two years of life: 25.0%, 95% CI: (22.3, 27.7).

Rhinitis co-morbidities

At least one allergy-related co-morbidity was reported in 87.0%, and 42.9% had at least two co-morbidities (figure 2). Combined rhinoconjunctivitis, current asthma and atopic eczema were found in 11.8%. The most common co-morbidity to rhinitis was conjunctivitis symptoms (itchy or runny eyes) reported in 192/254 (75.6%)) whereas the latter was reported as the only symptom in 28 children. Compared to those without rhinitis, children with current rhinitis had a higher prevalence of current asthma (31.7%), wheeze (32.1%), current atopic eczema (30.3%), and more frequently BHR ($PD_{20} \leq 8$ μmol), severe BHR ($PD_{20} \leq 1$μmol) as well as higher DRS (all $p<0.001$, table 2). Children with combined rhinoconjunctivitis symptoms did not differ in other co-morbidities compared to children with rhinitis symptoms only. Children with rhinitis had similar rates of allergic sensitization whether they had asthma or not (78.5% and 70.0% respectively, $p=0.16$). The children with rhinitis, but no asthma, had more frequently BHR ($PD_{20} \leq 8$μmol) than children with neither asthma nor rhinitis (29.2% and 21.7%, respectively) and similar for severe BHR ($PD_{20} \leq 1$μmol) (10.1% and 4.7% and respectively), $p=0.001$.

Allergic sensitization and rhinitis phenotypes

Timothy grass, birch, dog, cat and mugwort were the most prevalent sensitizing inhalant allergens in the present study (figure 3a). Among children with rhinitis, 185 (72.8%) were sensitized to at least one inhalant allergen and the majority had mixed sensitization (64.9%), whereas 25.9% were sensitized to seasonal allergens only. Rhinitis children *with* compared to children *without* sensitization to inhalant allergens had significantly more often conjunctivitis (85.9 vs 47.8%, respectively, $p<0.001$), BHR and severe BHR and a positive EIB test (table 3). They were similar in relation to current asthma, wheeze, otitis media and atopic eczema (table 3).

The children who were sensitized to more than one allergen (polysensitization) (n=266) were twice as likely to have current rhinitis compared to the children who were monosensitized (n=83) (59.8% vs 31.1%, respectively, p<0.001). The 26 rhinitis children who were monosensitized were mostly sensitized to pollens; timothy grass (n=11), birch (n=10) and mugworth (n=2). More severe BHR (PD_{20}≤1) was common for children with polysensitization compared to children who were monosensitized (20.5% and 9.8%, respectively, p=0.005), and polysensitized children had also more often current asthma (29.0%) than the children with monosensitization (13.4%), p=0.005. Moreover, the likelihood of rhinitis increased with increasing number of allergic sensitizations (figure 3b), with odds ratio 1.45, 95% CI: (1.25, 1.68), p<0.001.

Specific triggers of rhinitis symptoms were not indentified in 55 children, and only five reported symptoms triggered by temperature alterations or physical activity. The remaining 194 children reported rhinitis symptoms triggered by allergens. They were more likely to be allergic sensitized (85.1%) and to suffer from conjunctivitis (88.7%) than those with unknown or non-allergen triggers (33.3%), (both p<0.001). There was also a tendency towards more wheeze in the children with allergen triggered rhinitis (p=0.057), but they were otherwise comparable in the other potential co-morbidities of rhinitis (all p>0.2, figure 4).

Overall, 178/254 (70.1%) reported rhinitis symptoms after exposure to pollen (125 to pollen only and 53 to pollen *and* furry pets), whereas 15 children reported symptoms only when exposed to furry pets. Children with both pollen and furry pets as perceived triggers had more frequently co-morbidities of BHR, asthma and wheeze compared to the children with symptoms only related to pollen (table 4). The majority of the children with pollen-triggered rhinitis had a mixed sensitization status (50.4%), whereas 28.8% were sensitized to seasonal allergens only and 18.4% were not sensitized to any inhalant allergens (table 4). As expected, most of the children with rhinitis related to pollen exposure only reported symptoms in spring/summer season (91.9%). In spite of less co-morbidities, they were more likely to have symptoms with moderate/severe impact on daily life activities (52.0%) compared to children with pollen and furry pets as perceived triggers (35.8%, p=0.048).

Regardless of rhinitis symptoms, the children with mixed sensitization had more frequently severe BHR (PD_{20}≤1) (23.5%) than the children with a seasonal allergen sensitization only (7.8%), p=0.001 and similar for DRS (GM: 3.15, (95% CI: 2.3, 4.24) for mixed *vs* 1.45 (1.02, 2.05) for seasonal sensitization, p=0.001). However, the children with sensitization to perennial allergens *only* did not differ in BHR (PD_{20} methacholine and DRS) from the children with a mixed sensitization (both p>0.1).

Medical treatment of rhinitis

Overall, 177/254 (69.7%) of the children with current rhinitis had received medical treatment due to rhinitis, most often systemic (n= 149, 58.7%) or topical (38.6%) antihistamines whereas 29.9% had used both types of antihistamines. Treatment with alternative or complementary medicine was reported in 18 children (7.0%), 14 with homeopathic medicine. The alternative medicine was mostly (n=16) used as complementary to traditional medicine.

DISCUSSION

Current rhinitis was reported in 25.0% of 10 year old children, and was associated with at least one allergy-related co-morbidity in 87.0%, and 42.9% had at least two co-morbidities. Most common were conjunctivitis, current asthma, and atopic eczema, but they were also likely to have current wheeze and BHR. Seventy-three per cent of the children with rhinitis were sensitized to at least one inhalant allergen and these children had more frequently conjunctivitis and BHR, but did not differ in other co-morbidities compared to the children without allergic sensitization.

As many as 11.8% of the children with current rhinitis (3.0% of all children in the present study) had all three co-morbidities of conjunctivitis, asthma and atopic eczema present, which are much higher than the reports from the ISAAC study (0.6 - 1.1% of 13-14 year old children from Europe) (22). For the rhinitis children in the present study, conjunctivitis was the most commonly reported co-morbidity by 75.5%. Thus rhinoconjunctivitis is likely to be one disease entity for the majority of children in this age group with allergic sensitization and rhinitis. The 25.0% prevalence of current rhinitis in the present study is in line with the findings of the ISAAC study, phase three (22) which reported symptoms of rhinitis in 23.4% - 31.2% of the 13-14 year old European children. Current nose *and* eye symptoms was reported for 9.8% - 15.2% (22) compared to 18.9% in the present study.

The link between asthma and allergic rhinitis is well established (9), and confirmed in the present study as almost one in three children (31.7%) with rhinitis had asthma. We also found that BHR was associated with rhinitis, as has previously been confirmed by others (23), but not different between children with rhinitis *with* and *without* conjunctivitis, as in line with the MAAS study (24). Although BHR is a feature of asthma, it is non-specific, and in the present study, BHR was associated with rhinitis also in children without current asthma. Moreover, because rhinitis and asthma often co-exist, co-morbidities and symptoms of chronic rhinitis may be attributed to a common airways disease starting at the nose and including asthma (1, 2). However, allergic sensitization in children with rhinitis was similar in children without (70.0%) and with asthma (78.5%) supporting the notion that the co-existence of asthma and rhinitis is not solely due to atopic predisposition in these two diseases (2).

Children in the present study with rhinitis were much more likely to suffer from conjunctivitis symptoms if they had allergic sensitization (85.9 vs 47.8%) most commonly reported as mixed sensitization. This is in line with another report (80% mixed sensitization) although including only subjects that suffered from allergic rhinitis alone (25). Conjunctivitis

was reported to be more common among subjects with seasonal (64.9%), than with perennial (46.4%) or mixed sensitization (47.9%) (25). In phase two of the ISAAC study (3) associations of rhinoconjunctivitis was more common with sensitization to seasonal allergens [population attributable fraction in affluent countries (36%)] than with perennial allergens (25%). However, mixed sensitization status was not assessed in the ISAAC study. Also, the present children with rhinitis symptoms reported to be triggered both by exposure to pollen and furry pets had more asthma, wheeze, and frequently BHR than children with symptoms reported to be triggered exclusively by pollen. Also in regard to sensitization status, children with a mixed sensitization had more severe BHR than children sensitized to seasonal allergens only. Others have also reported that the risk of asthma was higher for young adults with rhinitis when exposed to pollens or to animals compared to those with rhinitis related to only one of these triggers (2). Moreover, sensitization with concomitant exposure to perennial allergens is shown to enhance the development of BHR in children with asthma or wheeze (26, 27). Thus, in addition to assessing sensitization status, it is also important to know the actual trigger of the symptoms.

Pollen particles may be quite large, and are thus more easily trapped in the nose, inducing a local allergic response, without necessarily inducing symptoms in the lower respiratory system (28). This could explain why children with pollen-triggered rhinitis had less often co-morbidities of current asthma, wheeze and BHR compared to children with symptoms triggered by pollen and furry pets. The latter group also tended to have lower FEF_{50} % predicted and more often a positive EIB test. Severe symptoms on daily life activities were mostly reported by children who had pollen-triggered rhinitis, which may be explained by these patients being less well adapted to bouts of sneezing and runny nose during the pollen season, in comparison to the patients with more chronic nasal obstruction due to exposure to perennial allergens. Furthermore, the majority of children without detectable allergic sensitization still reported symptoms of rhinitis related to allergens (most often pollen). This is in line with reports on patients with non-allergic rhinitis who most frequently reported exposure to pollen (and house dust) as the cause of their rhinitis symptoms (29).

In the present study, 7.0% of the children with rhinitis had used alternative medicine, and most often homeopathic medicine. In a large study from Germany and Switzerland, allergic rhinitis was one of the most common diagnoses among patient using homeopathic medicine (30). The frequent use of alternative and complementary medicine in children with

rhinitis and asthma are most often due to dissatisfaction with conventional therapies and concerns about steroid side-effects (31).

Limitations of the study

The phrasing of the questions regarding rhinitis in the parental interview does not allow us to categorize the children according to the ARIA document (9) which recommends that the old terms 'seasonal' and 'perennial' should be replaced by the terms intermittent allergic rhinitis and persistent allergic rhinitis, respectively. Many of the children in Oslo with symptoms related to pollen would be in the category of persistent allergic rhinitis, due to the long pollen season [approximately 3-4 months (including 6-8 weeks for grass pollen)]. Patients with pollen-triggered rhinitis may have a greater bronchial hyperresponsiveness during spring/summer seasons (25), but in the present study, we aimed to avoid clinical examinations and methacholine challenge testing during the pollen season. We cannot exclude the presence of rhinosinusitis (infectious rhinitis) (9) in the children with unknown triggers of rhinitis, but the majority reported nevertheless their symptoms to be triggered by allergen exposure.

In conclusion, current rhinitis was reported in 25.0% of the 10-year-old children, and was associated with at least one allergy-related co-morbidity in 87.0%, as well as more often severe BHR and wheeze. Conjunctivitis and BHR was associated with rhinitis with allergic sensitization. The majority of the children had a mixed sensitization status, but symptoms triggered by pollen exposure only had the greatest impact on daily life activities.

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REFERENCES

1. Lack G. Pediatric allergic rhinitis and comorbid disorders. J Allergy Clin Immunol 2001: 108: S9-15.

2. Leynaert B, Neukirch C, Kony S, Guenegou A, Bousquet J, Aubier M, et al. Association between asthma and rhinitis according to atopic sensitization in a population-based study. J Allergy Clin Immunol 2004: 113: 86-93.

3. Weinmayr G, Forastiere F, Weiland SK, Rzehak P, Abramidze T, Annesi-Maesano I, et al. International variation in prevalence of rhinitis and its relationship with sensitisation to perennial and seasonal allergens. Eur Respir J 2008: 32: 1250-61.

4. Spector SL. Overview of comorbid associations of allergic rhinitis. J Allergy Clin Immunol 1997: 99: S773-S780.

5. Bousquet J, Gaugris S, Kocevar VS, Zhang Q, Yin DD, Polos PG, et al. Increased risk of asthma attacks and emergency visits among asthma patients with allergic rhinitis: a subgroup analysis of the investigation of montelukast as a partner agent for complementary therapy [corrected]. Clin Exp Allergy 2005: 35: 723-7.

6. Prieto JL, Gutierrez V, Berto JM, Camps B. Sensitivity and maximal response to methacholine in perennial and seasonal allergic rhinitis. Clin Exp Allergy 1996: 26: 61-7.

7. Bousquet J, Bachert C, Canonica GW, Casale TB, Cruz AA, Lockey RJ, et al. Unmet needs in severe chronic upper airway disease (SCUAD). J Allergy Clin Immunol 2009: 124: 428-33.

8. Johansson SG, Bieber T, Dahl R, Friedmann PS, Lanier BQ, Lockey RF, et al. Revised nomenclature for allergy for global use: Report of the Nomenclature Review Committee of the World Allergy Organization, October 2003. J Allergy Clin Immunol 2004: 113: 832-6.

9. Bousquet J, Khaltaev N, Cruz AA, Denburg J, Fokkens WJ, Togias A, et al. Allergic Rhinitis and its Impact on Asthma (ARIA) 2008 update (in collaboration with the World Health Organization, GA(2)LEN and AllerGen). Allergy 2008: 8-160.

10. Rondon C, Romero JJ, Lopez S, Antunez C, Martin-Casanez E, Torres MJ, et al. Local IgE production and positive nasal provocation test in patients with persistent nonallergic rhinitis. J Allergy Clin Immunol 2007: 119: 899-905.

11. Bousquet J, Fokkens W, Burney P, Durham SR, Bachert C, Akdis CA, et al. Important research questions in allergy and related diseases: nonallergic rhinitis: a GA2LEN paper. Allergy 2008: 63: 842-53.

12. Lodrup Carlsen KC, Haland G, Devulapalli CS, Munthe-Kaas M, Pettersen M, Granum B, et al. Asthma in every fifth child in Oslo, Norway: a 10-year follow up of a birth cohort study. Allergy 2006: 61: 454-60.

13. Haland G, Carlsen KCL, Sandvik L, Devulapalli CS, Munthe-Kaas MC, Pettersen M, et al. Reduced lung function at birth and the risk of asthma at 10 years of age. N Engl J Med 2006: 355: 1682-9.

14. Lodrup Carlsen KC, Pettersen M, Carlsen KH. Is bronchodilator response in 2-yr-old children associated with asthma risk factors? Pediatr Allergy Immunol 2004: 15: 323-30.

15. Bertelsen RJ, Lodrup-Carlsen K, Granum B, Carlsen KH, Haland G, Devulapalli CS, et al. Do allergic families avoid keeping furry pets? Indoor Air, doi:10.1111/j.1600-0668.2009.00640.x

16. Dreborg S, Frew A. Position Paper - Allergen Standardization and Skin-Tests. Allergy 1993: 48: 49-82.

17. Quanjer PH, Tammeling GJ, Cotes JE, Pedersen OF, Peslin R, Yernault JC. Lung volumes and forced ventilatory flows. Report Working Party Standardization of Lung Function Tests, European Community for Steel and Coal. Official Statement of the European Respiratory Society. Eur Respir J Suppl 1993: 16: 5-40.

18. Stanojevic S, Wade A, Cole TJ, Lum S, Custovic A, Silverman M, et al. Spirometry centile charts for young Caucasian children: the Asthma UK Collaborative Initiative. Am J Respir Crit Care Med 2009: 180: 547-52.

19. Crapo RO, Casaburi R, Coates AL, Enright PL, Hankinson JL, Irvin CG, et al. Guidelines for methacholine and exercise challenge testing-1999. This official statement of the American Thoracic Society was adopted by the ATS Board of Directors, July 1999. Am J Respir Crit Care Med 2000: 161: 309-29.

20. Carlsen KH, Engh G, Mork M. Exercise-induced bronchoconstriction depends on exercise load. Respir Med 2000: 94: 750-5.

21. O'Connor G, Sparrow D, Taylor D, Segal M, Weiss S. Analysis of dose-response curves to methacholine. An approach suitable for population studies. Am Rev Respir Dis 1987: 136: 1412-7.

22. Ait-Khaled N, Pearce N, Anderson HR, Ellwood P, Montefort S, Shah J. Global map of the prevalence of symptoms of rhinoconjunctivitis in children: The International Study of Asthma and Allergies in Childhood (ISAAC) Phase Three. Allergy 2009: 64: 123-48.

23. Cibella F, Cuttitta G, La GS, Hopps MR, Passalacqua G, Pajno GB, et al. Bronchial hyperresponsiveness in children with atopic rhinitis: a 7-year follow-up. Allergy 2004: 59: 1074-9.

24. Marinho S, Simpson A, Lowe L, Kissen P, Murray C, Custovic A. Rhinoconjunctivitis in 5-year-old children: a population-based birth cohort study. Allergy 2007: 62: 385-93.

25. Ciprandi G, Cirillo I, Vizzaccaro A, Tosca M, Passalacqua G, Pallestrini E, et al. Seasonal and perennial allergic rhinitis: is this classification adherent to real life? Allergy 2005: 60: 882-7.

26. Illi S, von Mutius E, Lau S, Niggemann B, Gruber C, Wahn U. Perennial allergen sensitisation early in life and chronic asthma in children: a birth cohort study. Lancet 2006: 368: 763-70.

27. Nelson HS, Szefler SJ, Jacobs J, Huss K, Shapiro G, Sternberg AL. The relationships among environmental allergen sensitization, allergen exposure, pulmonary function, and bronchial hyperresponsiveness in the Childhood Asthma Management Program. J Allergy Clin Immunol 1999: 104: 775-85.

28. Wilson AF, Novey HS, Berke RA, Surprenant EL. Deposition of inhaled pollen and pollen extract in human airways. N Engl J Med 1973: 288: 1056-8.

29. Rondon C, Dona I, Torres MJ, Campo P, Blanca M. Evolution of patients with nonallergic rhinitis supports conversion to allergic rhinitis. J Allergy Clin Immunol 2009: 123: 1098-102.

30. Witt CM, Ludtke R, Mengler N, Willich SN. How healthy are chronically ill patients after eight years of homeopathic treatment?--Results from a long term observational study. BMC Public Health 2008: 8:413.: 413.

31. Shenfield G, Lim E, Allen H. Survey of the use of complementary medicines and therapies in children with asthma. J Paediatr Child Health 2002: 38: 252-7.

Table 1 Questions related to symptoms, triggers and medical treatment of rhinitis from the structured parental interview at the 10-year follow-up (the questions are translated from Norwegian)

Interview questions	**Outcomes**
Has the child had any of the following symptoms during the last 12 months (without a cold): a) runny nose, b) blocked nose, c) sneezing, d) itchy/runny eyes?	Rhinitis (a, b and/or c) Conjunctivitis (d)
What is causing the eye/nose symptoms? a) Not known b) Dog c) Cat d) Other furry pets e) Other causes f) Pollen from trees g) Other pollens h) Temperature changes/physical activity	Unknown cause of symptoms (a) Symptoms related to furry pets (b,c, d) Symptoms related to pollens (f, g) Symptoms related to furry pets *and* pollens (b, c d *and* b, c) Symptoms related to other causes than allergens (e, h)
Does the child have symptoms of rhinitis during a) the whole year, b) spring/summer or c) autumn/winter	Time of year when symptoms occur
How much impact have these symptoms had on daily life activities? a) No b) A little c) Moderate d) Severe	Impact on daily activities
Has the child ever been given the diagnosis hayfever/allergic rhinitis?	Doctor diagnosed hayfever ever
What kind of treatment has the child received due to symptoms of rhinitis? a) none b) don't know c) topical antihistamines d) systemic antihistamines e) topical cortocisteroids f) systemic cortocisteroids g) sodium cromoglycate h) leukotriene antagonist i) immunotherapy j) homeopathy k) other alternative medicine	Received medical treatment due to rhinitis (b-i) Alternative medicine (j-k)

Table 2 Co-morbidities and clinical characteristics for children with and without current rhinitis at 10 years of age (per cent unless otherwise stated)

	No rhinitis	**Current rhinitis**	
	n=761(75%)	**n=254 (25%)**	**p-value**
Gender (boys)	52.3	59.4	**0.048**
Conjunctivitis	3.7	75.6	**<0.001**
Current asthma	10.3	31.7	**<0.001**
Current wheeze	7.6	32.1	**<0.001**
Otitis media last year	7.1	9.1	0.3
Atopic eczema			
-previous	12.8	20.1	**0.004**
-current	18.2	30.3	**<0.001**
FEV_1% predicted (mean (95% CI))	99.6 (98.9, 100.3)	99.4 (98.1, 100.6)	0.8
FEF_{50}% predicted (mean (95% CI))	98.8 (97.3, 100.4)	96.8 (94.3, 99.4)	0.2
PD_{20} methacholine			
≤1µmol	5.8	17.5	
> 1 - ≤8 µmol	21.5	30.6	**<0.001**
> 8 µmol	72.7	52.0	
DRS (GM (95%CI))	1.07 (0.96, 1.20)	2.63 (2.09, 3.31)	**<0.001**
Exercise induced bronchoconstriction	16.5	22.0	0.069
Sensitization to inhalant allergens			
- none	78.4	27.2	
- seasonal	7.4	18.9	**<0.001**
- perennial	6.2	6.7	
- seasonal and perennial (mixed)	8.0	47.2	
Sensitization to food allergens	8.8	37.3	**<0.001**
Total IgE (GM (95%CI))	35.7 (32.0, 39.7)	128.8 (105.4, 157.4)	**<0.001**

Table 3 Co-morbidities and characteristics reported causes of symptoms, duration, severity and rhinitis medication for children with non-allergic or allergic rhinitis (per cent unless otherwise stated)

	Rhinitis without sensitization to inhalant allergens, n=69	**Rhinitis with sensitization to inhalant allergens, n=185**	**p-value**
Gender (boys)	42.0	65.9	**0.001**
Conjunctivitis	47.8	85.9	**<0.001**
Current asthma	25.0	34.3	0.2
Current wheeze	23.5	35.3	0.08
Otitis media last year	5.8	10.3	0.3
Atopic eczema			
-previous	18.8	20.5	0.8
-current	27.5	31.4	0.6
FEV_1% predicted (mean (95% CI))	99.1 (96.8, 101.5)	99.5 (98.1, 100.6)	0.8
FEF_{50}% predicted (mean (95% CI))	97.2 (92.8, 101.5)	96.7 (93.6, 99.8)	0.9
PD_{20} methacholine			**<0.001**
≤1µmol	4.3	22.4	
> 1 - ≤8 µmol	24.6	32.8	
> 8 µmol	71.0	44.8	
DRS (GM (95%CI))	1.25 (0.89, 1.76)	3.44 (2.60, 4.54)	**<0.001**
Exercise induced bronchoconstriction	11.3	25.6	**0.030**

Table 4 Co-morbidities and clinical characteristics for children with symptoms of rhinitis triggered by pollen only or by pollen *and* furry pets (per cent unless otherwise stated)

	Symptoms related to pollen only (n=125)	**Symptoms related to pollen *and* furry pets (n=53)**	**p**
Gender (boys)	57.6	64.2	0.4
Conjunctivitis	89.6	88.7	0.9
Current asthma	28.5	47.1	**0.018**
Current wheeze	29.6	49.1	**0.013**
Otitis media last year	8.8	15.1	0.2
Atopic eczema			
-previous	17.6	22.6	0.8
-current	32.8	39.6	0.4
FEV_1% predicted (mean (95% CI))	99.8 (98.1, 101.4)	98.5(95.4, 101.6)	0.5
FEF_{50}% predicted (mean (95% CI))	98.6(94.8, 102.4)	91.4 (86.1, 97.8)	0.06
PD_{20} methacholine			
≤1 µmol	12.2	32.1	
> 1 - ≤8 µmol	27.6	32.1	**0.002**
> 8 µmol	60.2	35.8	
DRS (GM (95%CI))	2.2(1.6, 3.0)	5.0(2.9, 8.6)	**0.008**
Exercise induced bronchoconstriction	17.2	25.0	0.3
Sensitization to inhalant allergens			**<0.001**
- none	18.4	7.5	
- perennial	2.4	7.5	
- seasonal	28.8	7.5	
- seasonal and perennial (mixed)	50.4	77.4	
Total IgE (GM(95%CI))	142.5(109.8, 184.9)	283.9(172.8, 466.2)	**0.008**
Symptoms in spring/summer only*	91.9	49.1	**<0.001**
Moderate/severe mpact on daily life activities	52.0	35.8	**0.048**
Doctor-diagnosed hayfever	54.8	84.6	**<0.001**
Any rhinitis treatment	84.0	90.6	0.3

*The children who did not have symptoms in spring/summer season only had symptoms throughout the year (none had symptoms exclusively during winter season)

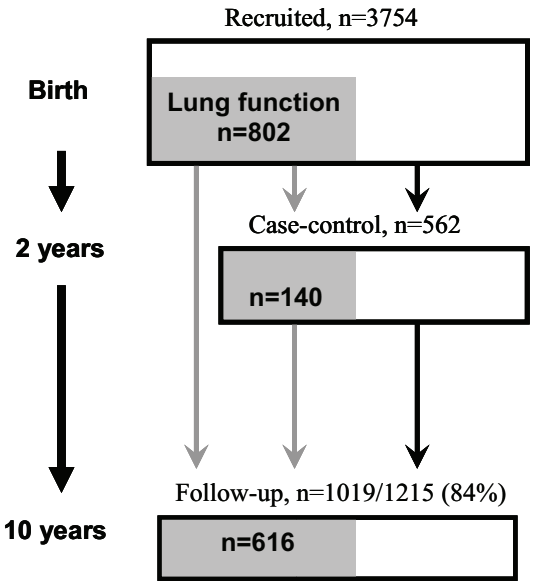

Figure 1 Illustration of The Environment and Childhood Asthma (ECA) Study design

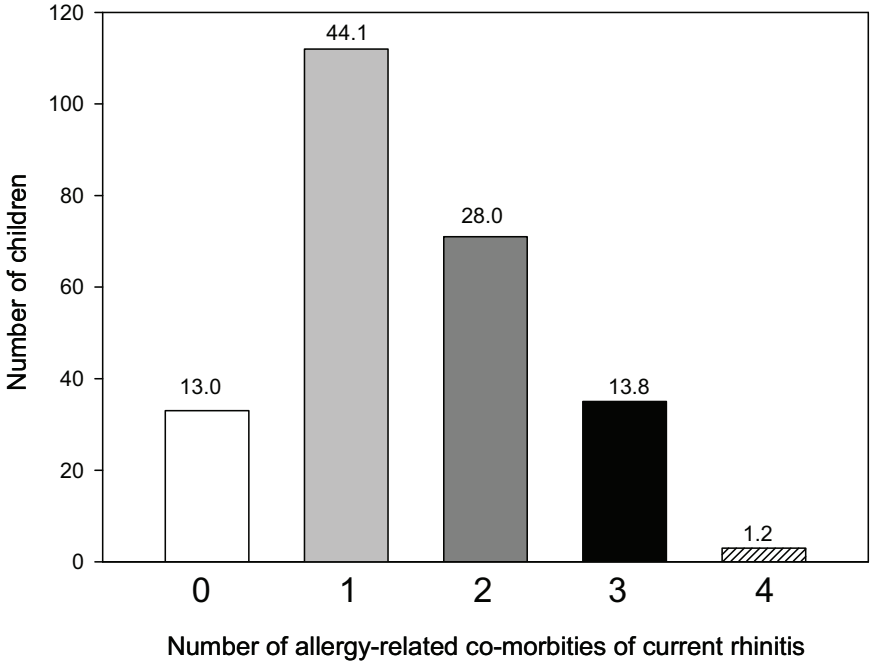

Figure 2 Bars referring to number of children with none, 1, 2, 3 or all 4 allergy-related co-morbidities of current rhinitis: conjunctivitis, current asthma, current atopic eczema and otitis media during the last year (per cent given above each bar).

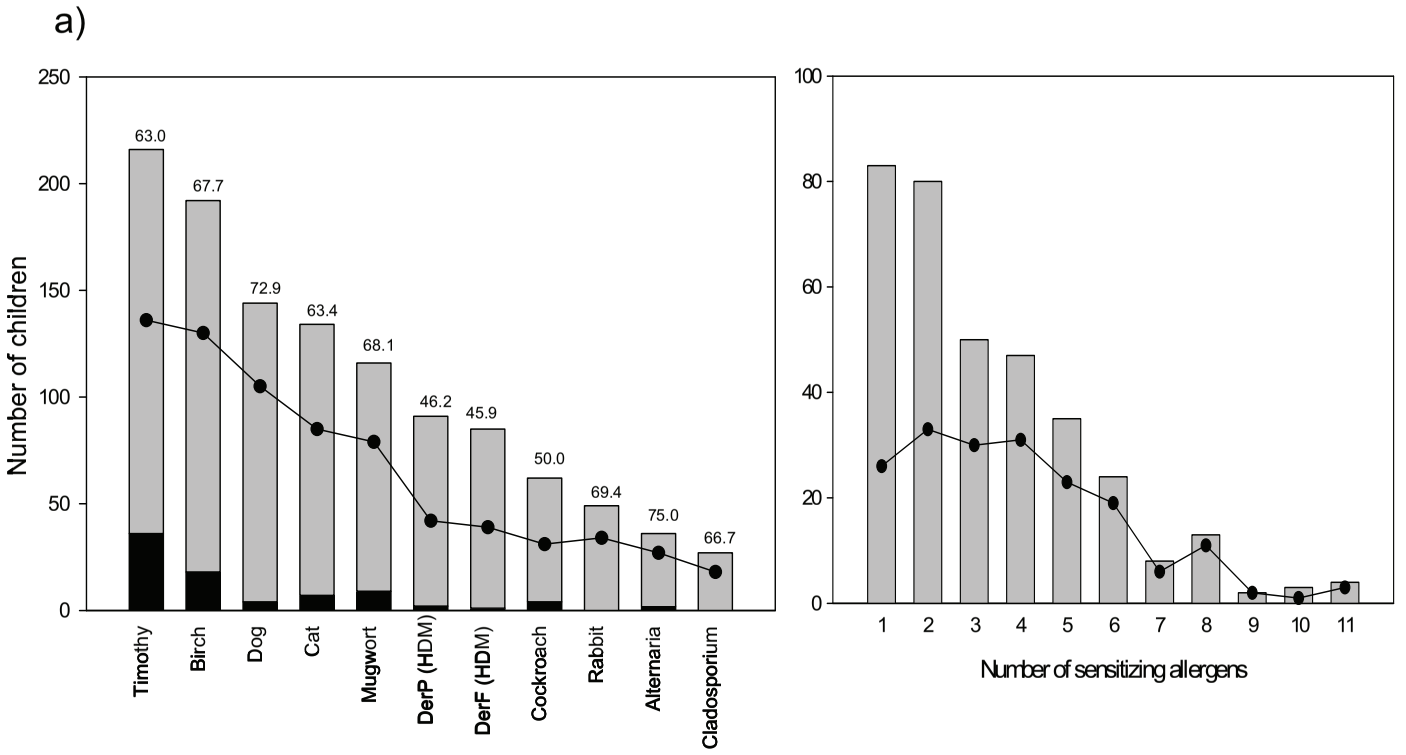

Figure 3a) Grey bars represents the number of children with sensitization to each of the specific inhalant allergens tested with increasing order from the most common (timothy grass) to the least common (Cladosporium), and the black bars represents the number of children with monosensitization. The dots represent the number of children for each specific sensitizing allergen who also have current rhinitis (the percentage of current rhinitis is given above each bar). **b)**Grey bars represents the number of children who were sensitized from 1 to all the 11 inhalant allergens tested. The dots represent the number of children who with current rhinitis

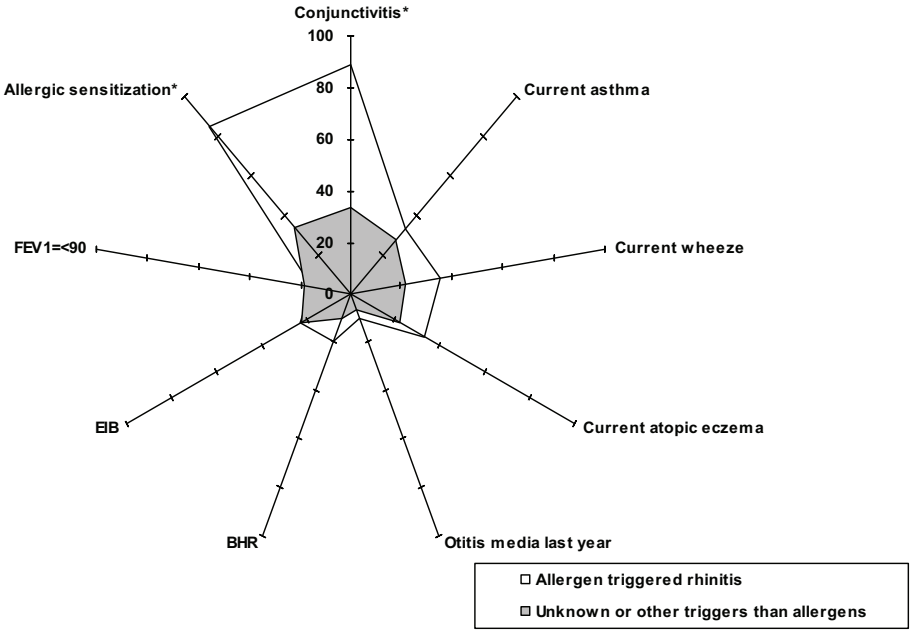

Figure 4 Radarplot of distribution of co-morbidities and clinical characteristics in children with rhinitis triggered by allergens (n=194) or unknown or other triggers than allergens (n=60). BHR presented as $PD_{20} \leq 1$ µmol methacholine. The scale gives the percentage of children with each co-morbidity or phenotype
* for $p < 0.001$, or otherwise no significant difference between the groups

IV

GENDER DIFFERENCES IN INDOOR ALLERGEN EXPOSURE AND ASSOCIATION WITH CURRENT RHINITIS

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Abstract

Background Differences between boys and girls in allergic manifestations are well known, and this difference is possibly not attributed to biological differences alone.

Objective We therefore investigated whether boys and girls could be exposed to different allergen levels at home and whether the indoor allergen levels could be differently associated with rhinitis in boys and girls at 10 years of age.

Methods Cat, dog and house dust mite allergen (HDM) levels in mattress dust and interview data regarding current allergic disease were retrieved for 797 10-year old children (360 girls) in The Environment and Childhood Asthma Study in Oslo.

Results Cat and dog allergen levels were highest for children with current pet ownership. However, also those who had previously kept pets or who had regular contact with pets outside their home had higher allergen levels compared to those who had never owned pets. Girls had higher levels of cat and dog allergens in their mattress dust compared to boys, regardless of pet ownership, whereas no difference was seen for HDM allergen (Der p1) levels. Of the 190 (23.8%) children reporting current rhinitis, 144 were sensitized to at least one allergen. The adjusted odds ratio for current rhinitis increased with 1.20 (95% CI: 1.01, 1.42) per 1 µg/g dust increase in Der p1 levels for girls (p=0.037), but not for boys (p=0.91). The children with unknown triggers of rhinitis had significantly higher dog allergen concentrations in their mattress dust (GM: 0.67 (95% CI: 0.37, 1.22) µg/g dust) compared to the children with perceived rhinitis triggers (0.34 (0.25, 0.46)), p=0.050.

Conclusion Girls had higher levels of pet allergens in mattress dust compared to boys, whereas no difference was seen for Der p1 allergen levels. Nevertheless, increasing levels of Der p1 significantly increased the risk of current rhinitis, but in girls only.

Keywords: gender, house dust mite, indoor allergens, pet keeping, rhinitis

Introduction

Rhinitis is one of the atopic manifestations in children, associated with asthma [1, 2] and bronchial hyperresponsiveness (BHR) [3], but can be present independently of allergic sensitization. Exposure to indoor allergens such as animal dander and house dust mite (HDM) increases the risk for both asthma and rhinitis [4]. Indoor allergens may exacerbate symptoms and increase BHR also in non-asthmatic subjects with allergic rhinitis [4, 5]. Thus, allergen avoidance is important to reduce symptoms in sensitized individuals. The observed inverse association between pet keeping and sensitization to pet allergens [6] has been attributed to pet avoidance in allergic families [6, 7]. We have previously reported that families of children with doctor confirmed allergic rhinitis were less likely to acquire cats and dogs during the 10 first years of life [8]. However, to be aware of all allergen exposure is virtually impossible since pet allergens are ubiquitously present in public areas and passively transferred to homes without pets [9, 10]. Moreover, even low allergen levels may induce symptoms in sensitized patients, as was shown by demonstrating nasal symptoms in patients challenged with concentrations of cat allergen (Fel d1) comparable to allergen levels in homes without cats [11].

In many populations mites are common with a corresponding high population prevalence of HDM sensitization [12]. In other populations, such as many areas of Scandinavia, HDM allergens are rarely found in the mattresses of children's bed [13, 14]. Exposure to HDM is frequently a low grade chronic exposure occurring overnight in bed, and controlled trials of primary avoidance of HDM have been relatively unsuccessful [15]. However, even low HDM allergen (Der p1) levels have been associated with allergic sensitization [14, 16], but also with nasal symptoms [17] and asthma exacerbation [18] in patients without detectable serum specific immunoglobulin E (s-IgE) to HDM.
In pre-puberty, boys have more often allergic disease than girls, whereas the gender association is reversed after puberty with asthma more commonly found in young women [19]. However, boys and girls differ not only in biology, but also in their behaviour and interests, which may affect the level of allergen exposure. Differences in risk factors and susceptibility to pet exposure have been reported [20]. In the present cohort, families in which the index child was a girl were more likely to acquire furry pets than families in which the index child was a boy [8].

It is not clear to what extent gender interacts with the associations between allergen exposure and allergic disease in school age. The aims of the present study were therefore first to describe the level of exposure to indoor allergens for girls and boys, and secondly to assess

whether there was a gender interaction between indoor allergen levels and the association with rhinitis and BHR.

Material and methods

Subjects

The present study reports results from the 10-year follow-up of the birth cohort 'The Environment and Childhood Asthma Study' (ECA) initiated in Oslo in 1992-1993. In total 1019 (84%) of 1215 invited children participated. As previously described [21], the follow-up study included extended clinical examination and a parental structured interview. The parents of the 1019 participating children were asked to collect dust from the child's mattress. Of these, 154 did not want to collect a dust sample, 27 did not return the sample and 41 samples could not be analysed due to insufficient amounts of dust. The remaining 797 children were included in the present study.

The 797 children with a mean age of 10.6 years (min: 8.8, max: 12.3) had less often cats at home (18% vs 26%, p=0.007), less often parents who smoked (38% vs 47%, p=0.02), and less often parents with higher education than the 222 non-included children (no dust sample). They were otherwise comparable in regard to the children's and the parents' allergic disease, family income, type of housing, and pet ownership (other than cats).

Written informed consent was obtained from the parents, and the study was approved by the Regional Committee for Ethics in Medical Research and the Norwegian Data Inspectorate and reported to the Norwegian Biobank Registry.

Dust sampling and extraction

The families were instructed not to change the linen on the child's bed for one week prior to the sampling of the mattress dust. The dust was collected by vacuuming the mattress for four minutes with vacuum cleaners equipped with a special dust collector (P-B Miljø A/S, Bjerringbro, Denmark) [22]. At the laboratory, the dust was vacuumed (sieved) through a filter with pore-size 0.3 µm, and the fine fraction of the dust was weighed and extracted in phosphate-buffered saline with 0.02% NaN_3 to a final concentration of 25 mg/ml and agitated in room temperature overnight. The extracted samples were then stored at -20ºC until analysis.

Analyses of cat, dog and mite allergens

The concentrations of the major cat (*Felis domesticus* Fel d1), dog (*Canis familiaris* Can f1) and mite (*Dermatophagoides pteronyssinus* Der p1) allergens were determined by enzyme-linked immunosorbent assay (ELISA) following the manufacturer's instructions (Indoor Biotechnologies, Clewyd, UK) [23] and as described in detail elsewhere [24]. Results were

given as µg allergen per gram dust. The detection limits of the ELISA were determined according to the standard curve for each run. Values below the detection limit were, for statistical purposes, given values of 2/3 of the detection limits: 0.12 µg/g for *Der p1*, 0.03 µg/g for *Can f1* and 0.20 µg/g for *Feld d1*. For the dog allergen assay 57 samples were below the detection limit, 215 samples for cat allergen and 697 for HDM allergen (Der p1).

Clinical examination at the 10-year follow-up

Two clinical examinations were performed 2-7 days apart and at least four weeks after any respiratory tract infection. The examinations included blood tests, skin prick tests for allergic sensitization, lung function by forced flow-volume measurements and bronchial hyperresponsiveness measured by methacholine challenge test and a treadmill exercise test.

Skin prick tests (SPT) were performed according to European standards [25] with the following standardized allergen extracts from ALK (Soluprick®, ALK-Abello, Denmark): house dust mites (*Dermatophagoides pteronyssinus* and *Dermatophagoides farinae*), German cockroach, dog, cat and rabbit, birch, timothy (grass) and mugwort pollen, moulds (*Cladosporium herbarium* and *Alternaria*), egg white, milk, peanut and codfish. SPT were considered positive with a wheal diameter at least 3 mm larger than the negative control (saline).

Specific serum IgE (sIgE) was measured with a radioallergosorbent fluorescence immunoassay (ImmunoCAP® system, Phadia, Uppsala, Sweden). Specific IgE was analysed with the same panel of allergens as for SPT, and considered positive when sIgE ≥0.35 kU/L.

Baseline spirometry was performed according to European standards [26] on a Sensor Medics Vmax 20c instrument (SensorMedics Diagnostics, Yorba Linda, CA, USA) prior to and after methacholine and exercise challenge tests on separate days. Short and long-acting beta-2 receptor agonists were withheld for at least 3 and 18 hours, respectively as were other relevant medications according to international guidelines prior to investigations [21].

The methacholine challenge test was measured as PD_{20} methacholine according to international guidelines [27], by inhalation of doubling doses of methacholine, nebulised by the Spira nebuliser (Spira Respiratory Care Center LtD, Hämeenlinna, Finland). The response was reported as the dose of methacholine that caused a fall in FEV_1 (forced expiratory volume in one second) of 20% from the (post-saline) baseline value (PD_{20} methacholine). A standardized exercise test [28] was performed on the second day by a 6-8 minutes treadmill run at 95% estimated maximum pulse level with 5.5% inclination. The exercise test was

considered positive for exercise-induced bronchoconstriction with a fall in FEV_1>10% of baseline FEV_1 measured 3-20 minutes after running had ceased.

Parental structured interview

The parental structured interview included central ISAAC questions related to airways symptoms of the child, including rhinitis. The questions related to rhinitis included perceived triggers of rhinitis (furry pets, pollens, temperature changes, physical activity or unknown), in which seasons the symptoms occurred (all year, spring/summer or autumn/winter) and the impact the rhinitis symptoms had on daily life activities (none, some, moderate or severe). Information about pet ownership and contact with pets outside the home was retrieved from the questions: "Do you currently keep a dog?" and "Is the child in regular contact with a dog?" The same questions were asked for cats and for other types of furry pets (rodents).

Definitions

Current rhinitis was present in a child reporting at least one of the following symptoms during the last 12 months (without a cold): runny nose, blocked nose, or sneezing. C*urrent conjunctivitis* required a report of itchy/runny eyes within the last 12 months.

Rhinitis with allergic sensitization was defined by symptoms of rhinitis combined with at least one positive SPT and/or sIgE to any of the *inhalant seasonal* (pollen or moulds) or *perennial* (mite, cat, dog, rabbit or cockroach) allergen.

BHR was defined as a methacholine dose-response slope (DRS) (higher DRS indicating more BHR) calculated from each individual as per cent decline in FEV_1 after the final dose of methacholine divided by the cumulative dose of methacholine inhaled [29].

Asthma was defined by the presence of at least two of the three criteria; asthma symptoms, doctor's diagnosis of asthma and use of asthma medication. *Current asthma* was defined as asthma plus at least one of the following criteria; asthma symptoms or use of asthma medication within the last 12 months and a positive exercise challenge test at the 10 year follow-up visit [21].

Statistical analyses

Categorical variables were compared using Pearson's chi-square test and linear-by-linear association for trends with more than two categories. One-way ANOVA was used to compare allergen concentrations (log-transformed and presented as geometric mean (GM) and 95% confidence intervals (95% CI)), and two-way ANOVA to adjust for pet keeping. To assess

whether gender interacted with the indoor allergen exposure levels, multivariate regression analyses were performed for rhinitis, DRS, impact on daily life activities and rhinitis with unknown rhinitis triggers. We also included current asthma and reported pet avoidance in the multivariate models as these have been confirmed to be associated with rhinitis for children in this cohort.. DRS was log-transformed due to skewed distribution, and due to the violation of normality and homoscedasticity assumptions. A robust regression was performed by using Huiber's method [30]. The result of the regression analysis was back-transformed for DRS. The final model for unknown rhinitis triggers was ascertained with the Akaike Information Criterion (AIC) [31]. The criterion for linearity for the logistic regression model, and the condition for normally distributed residuals of the dependent variables in the linear regression model, were both fulfilled. Cat, dog and HDM allergens were, therefore, used as continuous variables without log-transformation in the logistic and linear regression models. All models were tested for confounding and interaction. P-values ≤ 0.05 were considered significant. Statistical analyses were performed with Statistical Package for Social Sciences (SPSS version 14.0, SPSS Inc., Chicago, Illinois, USA). For the robust regression Number Cruncher Statistical System (NCSS version 2007, Kaysville, Utah, USA) was applied.

Results

Current rhinitis was found in 190/797 (23.8%) of the 10-year-old children. Boys had significantly more often current asthma, rhinitis, and wheeze as well as higher DRS compared to the girls (table 1). Also, the boys were significantly more often sensitized to perennial, seasonal and food allergens than the girls (table 1).

Cat and dog allergen levels in mattress dust

The highest cat and dog allergen levels were found in homes of cat-owners (18.2%) and dog-owners (14.3%); GM (95% CI): 37.9 (26.3, 54.7) and 32.2 (26.1, 44.8) µg allergen/g dust, respectively (figure 1). In mattresses of families who previously (but not currently) owned cats (38/640, 5.9%) or dogs (41/672, 6.1%), the cat and dog allergen levels were significantly higher than for those who had never kept pets (figure 1). The cat and dog allergen levels were also significantly higher in the mattresses of children with regular contact with cats or dogs outside their home compared to children with no ownership or regular contact with these animals (both $p<0.001$) (figure 1).

Girls compared to boys reported significantly more often cats at home (21.2% *vs* 15.9%) and contact with cats outside home (8.4% *vs* 6.7%), overall $p=0.03$. Whereas for cat ownership alone the difference was no statistically significant ($p=0.062$). No statistically significant gender difference was seen for dog ownership or contact (table 1), and neither for dog ownership alone, $p=0.4$. Nevertheless, the levels of both cat and dog allergens in mattress dust was significantly higher for girls compared to boys (table 1). The difference remained significant for cat ($p<0.001$) and dog allergen levels ($p=0.001$), after adjustment for pet contact and ownership.

Allergic sensitization and allergen exposure

Compared to non-sensitized children (any allergen), the 34.0% of the children sensitized to at least one inhalant allergen had significantly lower levels of cat allergens: GM (95% CI): 0.99 (0.77, 1.26) µg Fel d1/g dust for sensitized children *vs* 1.54 (1.27, 1.87) for non-sensitized children, $p=0.006$. The difference was also significant for dog allergen levels in mattress dust of sensitized children: GM: 0.41 (0.32, 0.53) µg Can f1/g dust vs non-sensitized children: 0.75 (0.62, 0.91), $p<0.001$ (table 2). Also significantly so after adjusting for dog ownership, whereas for cat allergen levels the difference was no longer significant after adjusting for cat ownership (table 2).

The Der p1 allergen levels were generally low in mattresses, and 87.7% of the samples had levels below the detection limit of the assay. Nevertheless, the geometric mean Der p1 allergen level in mattress dust of children sensitized to Der p1 (8.8%) was significantly higher than for children without Der p1 sensitization: 0.21 (0.16, 0.28) and 0.16 (0.15, 0.17) µg/g dust, respectively, p=0.004.

Allergen exposure, current rhinitis and BHR

Among girls and boys with current rhinitis, boys were more often sensitized to inhalant allergens (83.9%) than girls (62.5%) (p=0.001). However, boys and girls did not differ in reports of conjunctivitis symptoms, the types of triggers related to their symptoms or the impact the symptoms had on daily life activities (all p≥0.4).

Children with current rhinitis had significantly less often pets at home and lower pet allergen levels than among children without rhinitis, 0.97 (0.72, 1.29) *vs* 1.46 (1.22, 1.74) µg cat allergen/g dust for children with *vs* without current rhinitis, respectively (p=0.023), and similar for dog allergen: 0.40 (0.30, 0.52) *vs* 0.70 (0.58, 0.84) µg dog allergen /g dust, respectively (p=0.002) (table 2).

In the multivariate logistic regression model, gender interacted with asthma and HDM allergen exposure. The adjusted odds ratio for current rhinitis in girls increased with 1.20 (95% CI: 1.01, 1.42) per µg increase in Der p1 allergen level, p=0.037, whereas no association was seen for boys (p=0.91) (table 3). No significant association was seen for cat and dog allergen levels and current rhinitis symptoms.

In the multivariate linear regression model with logDRS as dependent variable, allergic sensitization interacted with dog allergen levels. DRS (back-transformed values) decreased with 0.99 (regression coefficient (*b*), (95% CI: 0.98, 1.00), p=0.004) per 1 µg increase in dog allergen levels, for children *with* allergic sensitization (table 4). The association was not significant for children without allergic sensitization (p=0.25).

HDM, cat and dog allergen levels in mattress dust were not associated with the impact the rhinitis symptoms had on daily life activities (none/some *vs* moderate/severe) (results not shown).

Cat and dog allergen exposure in relation to reported triggers of rhinitis

Children reporting at least one perceived rhinitis trigger (n=149) reported mostly symptoms related to allergen exposure (97.3%), most often pollen (62.0%) or both pollen *and* furry pets (28.3%), and only a few reported symptoms related to furry pets only (7.0%). Children with

unknown triggers (n=41) were more likely to have symptoms throughout the year (55.6%) compared to those with perceived rhinitis triggers (24.6%), p<0.001. The children with unknown triggers were also less likely to be allergically sensitized (34.1%) compared to those with perceived rhinitis triggers (87.2%), p<0.001.

The children with unknown triggers of rhinitis had significantly higher levels of dog allergen in their mattress dust [GM: 0.67 (95%CI: 0.37, 1.22) µg/g dust] compared to the children with perceived rhinitis triggers [0.34 (0.25, 0.46)], p=0.050 (two-way ANOVA). However, in the multivariate logistic regression model with indoor allergen levels, allergic sensitization and reported pet avoidance as potential explanatory variables, only Der p1 allergen levels in interaction with pet avoidance was significant determinants of rhinitis with unknown triggers. The adjusted odds ratio for rhinitis with unknown triggers increased with 2.52 (95% CI: 1.26, 5.03) per 1 µg increase in Der p1 allergen levels (p=0.01), but only for children *with* reported pet avoidance, whereas no association was seen for children *without* reported pet avoidance (p=0.82).

Discussion

In the present study, girls were exposed to higher levels of both cat and dog allergens than boys, a difference that could not be explained by pet ownership. Children with rhinitis had lower levels of pet allergen in the mattresses than children without rhinitis. HDM allergen was only detectable in 12% of the mattress dust samples. Nevertheless, increasing HDM allergen levels significantly increased the risk of current rhinitis, but in girls only, and were associated with rhinitis caused by unknown triggers in children with reported pet avoidance.

Higher cat and dog allergen levels in mattresses of girls compared to boys, also after adjustment for pet ownership, have to our knowledge not been reported previously. However, a recent study from Sweden reported more cat keeping among girls than boys, with a significant interactive effect on the risk for current wheeze for girls only [20]. Both studies may point to a greater inclination among girls to be with and cuddle pets. The higher *allergen* level in the present study may also be related to the tendency for girls to decorate their room with plush animals, fluffy pillows and carpets which may act as reservoirs for the allergens [32, 33]. Carpets on the bedroom floor were, in the present study, more often reported by the girls than the boys (13.2% vs 9.3%, respectively, p=0.08). Furthermore, associations between allergic sensitization in young children and soft toys in their bed have been reported by others [34], albeit without describing allergen levels or gender related differences.

Children with regular contact with pets or who were previous pet owners had higher allergen levels in their mattress dust compared to children who had never kept pets, which is consistent with earlier reports [35-37]. These findings illustrate the importance of allergen contamination on the clothes of individuals without pets at home [32] and the difficulty in reducing the allergen load even after removing furry pets [38]. The presence of pet allergens in homes without pets and even more the presence of HDM allergen, may render the patients unable to relate the allergen exposure to their symptoms. Thus, the association with higher dog allergen levels in mattress dust of children with unknown factors of rhinitis could potentially be a cause of their symptoms. It is also compatible with the finding that these children often had rhinitis symptoms throughout the year.

Increasing dog allergen levels were associated with reduced DRS (less BHR), which probably reflects the tendency to avoid pets if the child has rhinitis. We have previously reported more BHR among children *with* than *without* rhinitis (submitted manuscript). In addition, a doctor confirmed diagnosis of allergic rhinoconjunctivitis prior to the 10-year follow-up study was the strongest predictor for never having kept cats and dogs [8]. This may be the reason why the children with current rhinitis at 10 years of age were also found to have

lower pet allergen levels in their mattress dust compared to children without rhinitis. Moreover, in the multivariate analysis, reported pet avoidance, *but not* cat and dog allergen levels, was significantly associated with current rhinitis symptoms.

The interaction between reported pet avoidance and HDM (Der p1) allergen levels as determinant for rhinitis with unknown triggers is difficult to explain. The result is the opposite of what one would expect if the families with reported pet avoidance also were likely to make efforts to reduce the overall allergen load (such as regular vacuuming of the mattresses). However, even in intervention studies it has been difficult to reduce the HDM allergen levels [15].

The levels of Der p1 in the present study were low, but in line with previous reports from Norway [16, 39]. Nevertheless, the children with Der p1 sensitization had higher Der p1 levels in mattress dust compared to children without sensitization, as has also previously been described in other Norwegian [16] and Swedish [14] study populations. The association between allergen levels and sensitization was seen regardless of gender, whereas the association between mattress dust levels of Der p1 and current rhinitis was significant for girls only. The reason why girls, but not boys, should experience current rhinitis symptoms due to HDM allergen exposure is difficult to explain, but one possibility is that the effect of HDM allergen exposure on rhinitis symptoms for boys is masked by the strong association between asthma and rhinitis.. Nevertheless, our findings point to the importance of HDM allergen related symptoms, even for allergen levels that are much lower than has conventionally been assumed to be important for disease development.

Strengths and limitations of the present study

In the present study, the dust samples were sieved and the allergen concentrations were measured in the fine fraction of the dust. Allergens from cat and dog are found to mainly bind to particles that are part of the fine fraction of the dust [40], and the sieved dust are thus believed to be representative for the airborne component of the sample. HDM allergens, on the other hand, are more often associated with mite feces that are usually larger particles and less likely to be airborne. Thus, although previous reports from Norway also have reported few mattress dust samples with detectable HDM allergen levels [16, 39], the sieving of the dust samples may have reduced the quantity of HDM allergens that could be measured.

The ECA study is a well characterised birth cohort with a high follow-up rate and includes environmental and detailed clinical characteristics at several time points. The rhinitis definition was based upon ISAAC questionnaires, but with no clinical examination of the

nasal cavity. However, we included objective measures of airways inflammation such as exhaled nitric oxide, consistent with the rhinitis diagnosis in the same population (described in detail elsewhere) (In press, Pediatr Allergy Immunol).

In conclusion, girls had higher concentrations of both cat and dog allergens in their mattresses compared to boys. This was not caused by pet ownership, but may be due to behavioural differences between the genders. Even in our population with HDM detection in less than 20% of children, exposure to low levels of HDM allergen was associated with current rhinitis in girls and with rhinitis triggered by unknown factors in children with reported pet avoidance.

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References

1. Kurukulaaratchy RJ, Matthews S, Arshad SH. Defining childhood atopic phenotypes to investigate the association of atopic sensitization with allergic disease. *Allergy* 2005; 60:1280-6.

2. Lack G. Pediatric allergic rhinitis and comorbid disorders. *J Allergy Clin Immunol* 2001; 108:S9-15.

3. Cruz AA, Popov T, Pawankar R, Annesi-Maesano I, Fokkens W, Kemp J *et al.* Common characteristics of upper and lower airways in rhinitis and asthma: ARIA update, in collaboration with GA(2)LEN. *Allergy* 2007; 62 Suppl 84:1-41.:1-41.

4. Boulay ME, Boulet LP. The relationships between atopy, rhinitis and asthma: pathophysiological considerations. *Curr Opin Allergy Clin Immunol* 2003; 3:51-5.

5. Ciprandi G, Cirillo I, Vizzaccaro A, Tosca M, Passalacqua G, Pallestrini E *et al.* Seasonal and perennial allergic rhinitis: is this classification adherent to real life? *Allergy* 2005; 60:882-7.

6. Kerkhof M, Wijga AH, Brunekreef B, Smit HA, de Jongste JC, Aalberse RC *et al.* Effects of pets on asthma development up to 8 years of age: the PIAMA study. *Allergy* 2009; 64:1202-8.

7. Bornehag CG, Sundell J, Hagerhed L, Janson S. Pet-keeping in early childhood and airway, nose and skin symptoms later in life. *Allergy* 2003; 58:939-44.

8. Bertelsen R, Lodrup-Carlsen K, Granum B, Carlsen KH, Haland G, Devulapalli CS, Munthe-Kaas M, Mowinckel P, Lovik M. Do allergic families avoid keeping furry pets? Indoor Air . doi:10.1111/j.1600-0668.2009.00640.x

9. Instanes C, Hetland G, Berntsen S, Lovik M, Nafstad P. Allergens and endotoxin in settled dust from day-care centers and schools in Oslo, Norway. *Indoor Air* 2005; 15:356-62.

10. Perzanowski MS, Ronmark E, Nold B, Lundback B, Platts-Mills TA. Relevance of allergens from cats and dogs to asthma in the northernmost province of Sweden: schools as a major site of exposure. *J Allergy Clin Immunol* 1999; 103:1018-24.

11. Bollinger ME, Eggleston PA, Flanagan E, Wood RA. Cat antigen in homes with and without cats may induce allergic symptoms. *J Allergy Clin Immunol* 1996; 97:907-14.

12. Erwin EA, Custis N, Ronmark E, Wickens K, Sporik R, Woodfolk JA *et al.* Asthma and indoor air: contrasts in the dose response to cat and dust-mite. *Indoor Air* 2005; 15 Suppl 10:33-9.:33-9.

13. Nafstad P, Oie L, Mehl R, Gaarder PI, Lodrup-Carlsen KC, Botten G *et al.* Residential dampness problems and symptoms and signs of bronchial obstruction in young Norwegian children. *Am J Respir Crit Care Med* 1998; 157:410-4.

14. Warner AM, Bjorksten B, Munir AK, Moller C, Schou C, Kjellman NI. Childhood asthma and exposure to indoor allergens: low mite levels are associated with sensitivity. *Pediatr Allergy Immunol* 1996; 7:61-7.

15. Gotzsche PC, Johansen HK. House dust mite control measures for asthma: systematic review. *Allergy* 2008; 63:646-59.

16. Dotterud LK, Van TD, Kvammen B, Dybendal T, Elsayed S, Falk ES. Allergen content in dust from homes and schools in northern Norway in relation to sensitization and allergy symptoms in schoolchildren. *Clin Exp Allergy* 1997; 27:252-61.

17. Huggins KG, Brostoff J. Local production of specific IgE antibodies in allergic-rhinitis patients with negative skin tests. *Lancet* 1975; 306:148-50.

18. Gent JF, Belanger K, Triche EW, Bracken MB, Beckett WS, Leaderer BP. Association of pediatric asthma severity with exposure to common household dust allergens. *Environ Res* 2009; 109:768-74.

19. Almqvist C, Worm M, Leynaert B. Impact of gender on asthma in childhood and adolescence: a GA2LEN review. *Allergy* 2008; 63:47-57.

20. Bjerg A, Sandstrom T, Lundback B, Ronmark E. Time trends in asthma and wheeze in Swedish children 1996-2006: prevalence and risk factors by sex. *Allergy* 2009.

21. Lodrup Carlsen KC, Haland G, Devulapalli CS, Munthe-Kaas M, Pettersen M, Granum B *et al.* Asthma in every fifth child in Oslo, Norway: a 10-year follow up of a birth cohort study. *Allergy* 2006; 61:454-60.

22. Dreborg S, Einarsson R, Lau S, Munir AK, Wahn U. Dust sampling for determination of allergen content. *Allergy* 1995; 50:188-9.

23. Chapman MD, Heymann PW, Wilkins SR, Brown MJ, Platts-Mills TA. Monoclonal immunoassays for major dust mite (Dermatophagoides) allergens, Der p I and Der f I, and quantitative analysis of the allergen content of mite and house dust extracts. *J Allergy Clin Immunol* 1987; 80:184-94.

24. Bertelsen RJ, Lødrup Carlsen KC, Carlsen K-H, Granum B, Doekes G, Håland G, Mowinckel P, Løvik M. Childhood asthma and early life exposure to indoor allergens, endotoxin and ß(1,3)-glucans. Clin Exp Allergy. doi:10.1111/j.1365-2222.2009.03424.x

25. Dreborg S, Frew A. Position Paper - Allergen Standardization and Skin-Tests. *Allergy* 1993; 48:49-82.

26. Quanjer PH, Tammeling GJ, Cotes JE, Pedersen OF, Peslin R, Yernault JC. Lung volumes and forced ventilatory flows. Report Working Party Standardization of Lung Function Tests, European Community for Steel and Coal. Official Statement of the European Respiratory Society. *Eur Respir J Suppl* 1993; 16:5-40.

27. Crapo RO, Casaburi R, Coates AL, Enright PL, Hankinson JL, Irvin CG *et al.* Guidelines for methacholine and exercise challenge testing-1999. This official statement

of the American Thoracic Society was adopted by the ATS Board of Directors, July 1999. *Am J Respir Crit Care Med* 2000; 161:309-29.

28. Carlsen KH, Engh G, Mork M. Exercise-induced bronchoconstriction depends on exercise load. *Respir Med* 2000; 94:750-5.

29. O'Connor G, Sparrow D, Taylor D, Segal M, Weiss S. Analysis of dose-response curves to methacholine. An approach suitable for population studies. *Am Rev Respir Dis* 1987; 136:1412-7.

30. Hamilton L. Regression with Graphics: A Second Course in Applied Statistics. Pacific Grove, California: Brooks/Cole Publishing Company, 1991.

31. Faraway JJ. Extending the Linear Model with R: Genralized Linear, Mixed effects and Nonparametric Regression Models. Boca Raton, FL: Chapman & Hall/CRC, 2006.

32. Almqvist C. High allergen exposure as a risk factor for asthma and allergic disease. *Clin Rev Allergy Immunol* 2005; 28:25-41.

33. de Blay F, Chapman MD, Platts-Mills TA. Airborne cat allergen (Fel d I). Environmental control with the cat in situ. *Am Rev Respir Dis* 1991; 143:1334-9.

34. de Bilderling G, Mathot M, Agustsson S, Tuerlinckx D, Jamart J, Bodart E. Early skin sensitization to aeroallergens. *Clin Exp Allergy* 2008; 38:643-8.

35. Almqvist C, Egmar AC, Hedlin G, Lundqvist M, Nordvall SL, Pershagen G *et al.* Direct and indirect exposure to pets - risk of sensitization and asthma at 4 years in a birth cohort. *Clin Exp Allergy* 2003; 33:1190-7.

36. Fahlbusch B, Gehring U, Richter K, Wichmann HE, Heinrich J. Predictors of cat allergen (Fel d 1) in house dust of German homes with/without cats. *J Investig Allergol Clin Immunol* 2002; 12:12-20.

37. Gehring U, Triche E, van Strien RT, Belanger K, Holford T, Gold DR *et al.* Prediction of residential pet and cockroach allergen levels using questionnaire information. *Environ Health Perspect* 2004; 112:834-9.

38. Woodcock A, Custovic A. ABC of allergies. Avoiding exposure to indoor allergens. *BMJ* 1998; 316:1075-8.

39. Gaarder PI. Mite-allergen levels in Norwegian homes. *Allergy* 1998; 53:58-9.

40. Ormstad H, Johansen BV, Gaarder PI. Airborne house dust particles and diesel exhaust particles as allergen carriers. *Clin Exp Allergy* 1998; 28:702-8.

Table 1 Characteristics and allergen levels in mattress dust (reported as µg allergen/g dust) of all included children and differences between boys and girls (percent or otherwise stated)

	All children (n=797)	Girls (n=360)	Boys (n=437)	*P* (girls vs boys)
Age (mean (min, max))	10.6 (8.8, 12.3)	10.6 (8.8, 12.3)	10.6 (9.0, 12.3)	0.99
Current asthma	15.0	10.6	18.7	0.002
Current wheeze	13.6	9.8	16.8	0.004
Current rhinitis	23.8	20.1	27.1	0.021
DRS (GM (95% CI))	1.37(1.22-1-54)	1.12(0.95-1.33)	1.61(1.36-1.90)	0.003
Sensitization to at least one allergen	34.0	25.3	41.2	<0.001
food allergens*	16.6	11.7	20.5	0.001
seasonal allergens**	27.9	19.5	34.8	<0.001
perennial allergens†	23.7	16.4	29.7	<0.001
house dust mite allergens‡	9.0	6.7	11.1	0.04
cat allergen	12.8	8.6	16.2	0.001
dog allergen	13.7	8.1	18.3	<0.001
Pet ownership and allergen exposure				
Dog allergen (GM (95% CI))	0.61(0.53, 0.72)	0.78(0.62, 0.98)	0.50(0.41, 0.62)	0.006
Dog at home	14.3	15.4	13.4	
Contact with dogs	11.4	11.5	11.3	0.4[#]
Cat allergen (GM (95% CI))	1.32(1.14, 1.54)	1.93(1.50, 2.47)	0.97(0.81, 1.17)	<0.001
Cat at home	18.2	21.1	15.9	
Contact with cats	7.5	8.4	6.7	0.03[#]
Mite allergen[§] (GM (95% CI))	0.16(0.15, 0.17)	0.16(0.15, 0.17)	0.16(0.15, 0.18)	0.6
Reported pet avoidance	33.7	31.4	35.6	0.2

[*]Egg white, milk, peanut and/or codfish..
**Pollens and/or moulds.
[†]HDM, cat, dog, rabbit and/or cockroach.
[‡]Der p1 and/or Der f1.
[#]Overall p-values for pet contact or ownership.
[§]Der p1.

Table 2 Association between cat and dog ownership and allergen levels for children with and without allergic sensitization and current rhinitis

		Cat ownership			Cat allergen		
		n	(%)	*P*	(GM (95% CI))	*P*	*P* *
Inhalant sensitization**	No	523	20.5		1.54 (1.27, 1.87)		
	Yes	269	13.8	**0.02**	0.99 (0.77, 1.26)	**0.006**	0.164
Cat sensitization	No	690	19.0		1.34 (1.14, 1.58)		
	Yes	102	12.7	0.1	1.18 (0.75, 1.86)	0.6	0.4
Current rhinitis	No	21.3	21.3		1.46 (1.22, 1.74)		
	Yes	8.4	8.4	**<0.001**	0.97 (0.72, 1.29)	**0.023**	0.4
		Dog ownership			Dog allergen		
		n	(%)	*P*	(GM (95% CI))	*P*	*P* *
Inhalant sensitization**	No	523	15.9		0.75 (0.62, 0.91)		
	Yes	269	11.2	0.07	0.41 (0.32, 0.53)	**<0.001**	**<0.001**
Dog sensitization	No	681	15.1		0.68 (0.57, 0.80)		
	Yes	109	9.2	0.1	0.32 (0.22, 0.47)	**0.001**	**0.002**
Current rhinitis	No	601	16.0		0.70 (0.58, 0.84)		
	Yes	190	8.9	**0.02**	0.40 (0.30, 0.52)	**0.002**	0.068

*Adjusted for cat or dog ownership.
** Any inhalant allergen

Table 3 Variables associated with current rhinitis. Due to significant gender interaction, the results are given separately for boys and girls.

Variables associated with current rhinitis	Boys (n=467)		Girls (n=360)	
	aOR(95% CI)	*P*	aOR(95% CI)	*P*
Allergic sensitization	13.36 (7.46, 23.92)	<0.001	8.70 (4.69, 16.13)	<0.001
Current asthma	2.76 (1.46, 5.22)	0.006	0.66 (0.26, 1.67)	0.38
HDM allergen (1µg/g dust)	1.00 (0.94, 1.06)	0.91	1.20 (1.01, 1.42)	0.037
Reported pet avoidance	2.15 (1.24, 3.72)	0.002	8.70 (4.69, 16.13)	<0.001

aOR = adjusted odds ratio. Cat and dog allergen levels was not significant determinants of current rhinitis in the multivariate models

Table 4 Linear regression analyses of determinants of DRS (higher DRS indicates more BHR) in 10-year old children. Due to significant interaction between allergic sensitization and dog allergen levels, results are given separately according to allergic sensitization (to any inhalant allergen)

Variables associated with increase in DRS	No allergic sensitization			Allergic sensitization		
	b	95% CI	*P*	*b*	SE	*P*
Intercept	0.12			0.55		
Dog allergen (1µg/g dust)	1.00	(1.00, 1.00)	0.25	0.99	(0.98, 1.00)	0.004
Current asthma	1.26	(1.03, 1.53)	0.02	2.06	(1.58, 2.68)	<0.001
Current rhinitis	1.15	(0.94, 1.41)	0.18	1.26	(1.00, 1.59)	0.055
Reported pet avoidance	1.12	(0.97, 1.29)	0.12	1.19	(0.94, 1.50)	0.15

b, regression coefficient. Gender, HDM and cat allergen exposure was not significant determinants for DRS

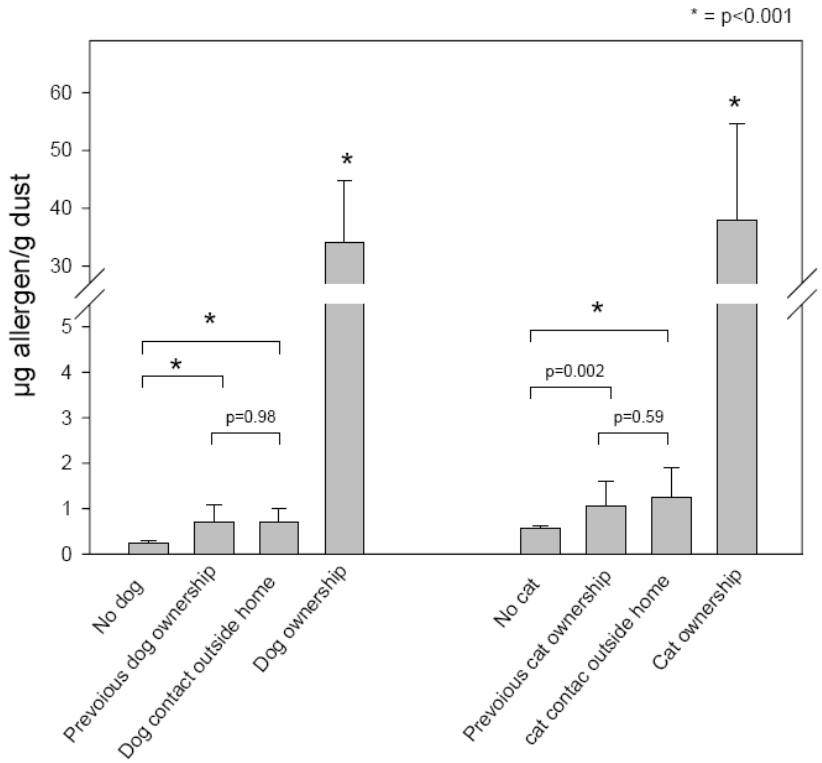

Figure 1: Differences in dog allergen levels [geometric mean (bars) with 95% confidence interval (CI)] between children with no dog contact or ownership ever, previous dog ownership, contact with dogs outside home and current dog ownership, and similar for cat allergens.
* *P* <0.001

Appendix A: Parts of questionnaire at birth (in Norwegian)

II: OPPLYSNINGER OM BOLIGEN OG MILJØET RUNDT BOLIGEN

12. Hva slag bolig bor du i?

- ☐ Enebolig
- ☐ Horisontalt delt bolig, første etasje
- ☐ Horisontalt delt bolig, annen etasje
- ☐ Rekkehus/vertikaldelt bolig
- ☐ Blokk/leiegård/terassehus, hvilken etasje_____

13. Hvor mange rom har boligen?
(ta med kjøkken, men ikke bad/WC og gang)_____

14. Hvilket år (omtrent) ble boligen bygget?_____

15. Hva slags oppvarming er det i boligen? (kryss av for alle typer som brukes)

- ☐ Sentralfyring/fjernvarme/radiatorer
- ☐ Parafinfyring i egen boligenhet
- ☐ Elektrisk
- ☐ Vedfyring i ovn
- ☐ Peis

16. Hvordan vil du samlet sett beskrive din boligstandard?

- ☐ Meget bra
- ☐ Bra
- ☐ Dårlig
- ☐ Meget dårlig

17. Hvilke rom har vegg til vegg tepper? (kryss av for alle aktuelle)

- ☐ Ingen
- ☐ Gang
- ☐ Alle soverom
- ☐ Noen soverom
- ☐ Oppholdsrom/stue

18. Hvilke husdyr har dere? (kryss av for alle aktuelle)

- ☐ Ingen
- ☐ Katt
- ☐ Hund
- ☐ Fugl
- ☐ Hamster/marsvin/rotte/mus/kanin
- ☐ Annet, hvilket_____

III: OPPLYSNINGER OM BILTRAFIKKEN DER DU BOR

19. Hvor langt er det fra boligen til nærmeste bilvei med gjennomgangstrafikk?

- ☐ Under 10 m
- ☐ 10-49 m
- ☐ 50-100 m
- ☐ Over 100 m

20. Hvor tett er trafikken ved boligen på det meste?

- ☐ Svært liten
- ☐ Liten
- ☐ Middels
- ☐ Stor
- ☐ Svært stor

21. Høres biltrafikk fra dagligrom/stue når vinduet er lukket?

- ☐ Ja, godt
- ☐ Ja, noe
- ☐ Nei, nesten ikke
- ☐ Nei, overhodet ikke

22. Er det andre forurensninsgkilder nær boligen enn veitrafikk?

- ☐ Ja, ----fyll ut----->
- ☐ Nei

- ☐ Industri
- ☐ Søppelforbrenning
- ☐ Annet, hva_____

IV: OPPLYSNINGER OM RØYKING OG ALKOHOL

23. Røyker du?

- ☐ Nei
- ☐ Ja, av og til
- ☐ Ja, daglig, hvor mange sigaretter om dagen?_____

(en pk. tobakk svarer til 50 sigaretter)

24. Endret du dine røykevaner under svangerskapet?

- ☐ Ja, ----fyll ut----->
- ☐ Nei
- ☐ Vet ikke

- ☐ Sluttet helt
- ☐ Røykte mer enn før
- ☐ Røykte mindre enn før
- ☐ Vet ikke

25. Røyker noen av de du bor sammen med?

☐ Nei
☐ Ja, av og til
☐ Ja, daglig. Dersom barnets far røyker, hvor mye røyker han?
(antall sigaretter pr. dag) _____
Dersom andre røyker, hvor mye røyker de tilsammen?
(antall sigaretter pr. dag) _____

26. Røykes det inne hos dere?

☐ Ja ----------> Blir det tatt spesielle hensyn? ☐ ja ☐ nei
☐ Nei

27. Nedenfor er listet opp en del hensyn som en kan ta når en røyker inne. Kryss av for alle de hensyn som blir tatt hjemme hos deg:

☐ Røyker ikke på soverom
☐ Røyker ikke i daglige oppholdsrom
☐ Lufter alltid når det blir røykt
☐ Røyker bare på spesielle steder
☐ Røyker bare på bestemte tidspunkt
☐ Annet_____

28. Hvor ofte drakk du alkohol i første halvdel av svangerskapet?

☐ Aldri
☐ En sjelden gang
☐ Ca. en gang pr. uke
☐ Oftere enn en gang pr. uke

29. Hvor ofte drakk du alkohol i siste halvdel av svangerskapet?

☐ Aldri
☐ En sjelden gang
☐ Ca. en gang pr. uke
☐ Oftere enn en gang pr. uke

30. Har du drevet med regelmessig fysisk trening mens du var gravid?

☐ Ja, ----fyll ut-----> 1-3 måned, ca. _____ ganger hver uke
☐ Nei 4-6 måned, ca. _____ ganger hver uke
7-9 måned, ca. _____ ganger hver uke

V: OPPLYSNINGER OM FAMILIENS HELSE

31. Har du, barnets far eller barnets søsken (hel eller halvsøsken) nå eller tidligere hatt en eller flere av følgende sykdommer? (kryss av for de som er aktuelle, for søsken skriv antall)

☐ Ja ----------fyll ut----------->
☐ Nei

	Mor	Far	Antall søsken
Astma ---------->	☐	☐	____
Eksem ---------->	☐	☐	____
Høysnue ---------->	☐	☐	____
Elveblest ---------->	☐	☐	____
Allergisk sjokk---------->	☐	☐	____
Gjentatt pustebesvær---------->	☐	☐	____
Gjentatt lungebetennelse---->	☐	☐	____
Gjentatt falsk krupp ---------->	☐	☐	____
Gjentatt ørebetennelse ------>	☐	☐	____
Gjentatt bihulebetennelse---->	☐	☐	____
Sukkersyke ---------->	☐	☐	____
Urinveis - og nyresykdom -->	☐	☐	____
Hjertesykdom ---------->	☐	☐	____
Kreft---------->	☐	☐	____
Epilepsi ---------->	☐	☐	____
Immunsvikt---------->	☐	☐	____
Andre (hvilke)			
_____ ---------->	☐	☐	____
_____ ---------->	☐	☐	____

VI: OPPLYSNINGER OM FORHOLD UNDER SVANGERSKAPET

31. Hadde du et normalt svangerskap?

☐ Ja
☐ Nei, hvilke komplikasjoner hadde du?_____

32. Brukte du riedempende medisiner?

☐ Ja
☐ Nei

33. Brukte du noen andre medisiner i svangerskapet?

☐ Ja ----fyll ut----->
☐ Nei

Hvilke, og når i svangerskapet	
Navn	tidsrom
_____	_____
_____	_____
_____	_____

Fars yrke

41. Er han yrkesaktiv?

- ☐ Ja, ----fyll ut-----> ☐ Heltid
- ☐ Nei ☐ Deltid, fast, antall timer pr. uke_____
- ☐ Vet ikke ☐ Deltid, uregelmessig, ca. timer pr. uke_____

42. Er han student ved siden av å være yrkesaktiv

- ☐ Ja
- ☐ Nei
- ☐ Vet ikke

43. Hvis han ikke er yrkesaktiv nå, hvilken gruppe passer han best i?
(kryss av for den viktigste)

- ☐ Student
- ☐ Hjemmearbeidende
- ☐ Arbeidsledig
- ☐ Uføretrygdet/under attføring
- ☐ Annet _____

44. Dersom han er/har vært yrkesaktiv, hva er/var hans yrke?_____

Din utdannelse

45. Hvilken utdanning har du fullført?

- ☐ 9-årig skole, eventuelt med et 10 år
- ☐ 1-2 årig folkehøyskole eller lignende
- ☐ Videregående skole/gymnas
- ☐ Videregående, yrkes-faglig studieretning:
  hvilken utdannelse:_____
  antall år:_____
- ☐ Høyskole: hvilken utdannelse:_____
  antall år:_____
- ☐ Universitet: hvilken utdannelse:_____
  antall år:_____
- ☐ Annet: hvilken utdannelse:_____
  antall år:_____

Fars utdannelse

46. Hvilken utdannelse har barnets far fullført? (kryss av for høyeste utdanning)

- ☐ 9-årig skole, eventuelt med et 10 år
- ☐ 1-2 årig folkehøyskole eller lignende
- ☐ Videregåendeskole/gymnas
- ☐ Videregående, yrkes-faglig studieretning:
  hvilken utdannelse_____
  antall år:_____
- ☐ Høyskole hvilken utdannelse:_____
  antall år:_____
- ☐ Universitet hvilken utdannelse:_____
  antall år:_____
- ☐ Annet hvilken utdannelse:_____
  antall år:_____
- ☐ Vet ikke

Inntekt og øvrige forhold

47. For deg som bor sammen med barnets far:
Hvor stor var felles bruttoinntekt (inntekt før skatt) siste år?

- ☐ Under 100 000
- ☐ 100-199 000
- ☐ 200-299 000
- ☐ 300-500 000
- ☐ Over 500 000

48. For deg som bor alene:
Hvor stor var din bruttoinntekt (inntekt før skatt) siste år?

- ☐ Under 100 000
- ☐ 100-200 000
- ☐ 200-300 000
- ☐ Over 300 000

49. Har du/dere ekstra arbeid med kronisk syke barn eller pleietrengende/kronisk syke pårørende?

- ☐ Ja, ----fyll ut-----> Vanligvis ca _____ timer i uken
- ☐ Nei

VIII: OPPLYSNINGER OM LIVSSITUASJONEN

De følgende spørsmål skal du besvare ved å merke av på linjen med et kryss der hvor det passer best for din situasjon.

50. Hvor lett vil det være å få noen til å se etter det nyfødte barnet ditt hvis du må gjøre et ærend en formiddag?

1 _____ 10

meget lett helt umulig

51. Hvor lett vil det være å få barnevakt til det nyfødte barnet en kveld? (se bort fra barnets far)

1 _____ 10

meget lett helt umulig

52. Hvor lett vil det være å kontakte slektninger eller venner ved spørsmål om stell av barnet?

1 _____ 10

meget lett helt umulig

53. Dersom du/dere fikk en uventet regning på 5 000.-, hvor lett ville det være å betale den i løpet av en uke?

1 _____ 10

meget lett helt umulig

54. Er du bekymret for forurensningen på stedet der du bor?

1 _____ 10

helt ubekymret meget bekymret

Appendix B: Parts of the interview form at age 10 years (in Norwegian)

Intervjuskjema Miljø og Barneastma - del 2
Familie/sosial/fritid

1.Kode ☐☐☐☐

2.Alder ☐☐ år ☐☐ mnd

3.Kjønn: ☐ 1.Gutt 2.Jente

Utfylt av: ☐☐

4.Dato første undersøkelse ☐☐.☐☐.☐☐ (dd mm åå)

5.Dato andre undersøkelse ☐☐.☐☐.☐☐ (dd mm åå)

6.Hvem følger ved intervju: ☐ 1.Mor 2.Far 3.Mor og far 4.Andre

Familie

7.Hvor mange søsken har barnet?

a ☐☐ Helsøsken

b ☐☐ Halvsøsken

8.Hvilken alder har barnets søsken som det bor sammen med nå?

a ☐☐ b ☐☐ c ☐☐ d ☐☐ e ☐☐

9.Hvem bor barnet sammen med >50% av tiden? ☐
1.Mor og far
2.Mor
3.Far
4.Likedelt mor og far
5.Andre

10.Hvor gammelt var barnet da mor og far evt. skilte lag?
☐☐ år

11.Hvor gamle var foreldrene da barnet ble født?

Mor ☐☐ år (a) Far ☐☐ år (b)

12.Hvor gammelt var barnet da andre evt. overtok omsorgen?
☐☐ år

13.Hva slags etnisk bakgrunn har foreldrene?

a Mor: ☐
b Far: ☐

1.Kaukasisk
2.Afrikansk
3.Asiatisk
4.Sør-Amerikansk
5.Samisk
6.Blandet
7.Annet

14.I hvilken kommune var barnets besteforeldre født?

Mors mor: _____ Mors far: _____

Fars mor: _____ Fars far: _____

15.Hva var familiens totale brutto yrkesinntekt siste år?

☐ 1.<350.000 2. 350-560.000 3. 560-750.000 4 >750.000

16.Hva er høyeste gjennomførte skolegang?

a Mor: ☐
b Far: ☐

1.Ikke gjennomført grunnskole
2.Grunnskole
3.Videregående
4.Høyskole/Universitet inntil 3 år
5.Høyskole/Universitet 4 år eller mer

17.Hva er foreldrenes grad av yrkesaktivitet?

	Mor	Far
Yrkesaktiv heltid	a ☐	f ☐
Yrkesaktiv deltid	b ☐	g ☐
Skoleelev/Student	c ☐	h ☐
Hjemmeværende	d ☐	i ☐
Uføretrygdet(helt el. delvis)	e ☐	j ☐

23227

Familiær sykehistorie

Kode ☐☐☐☐

18.Har mor eller far hatt, eller har de i dag følgende sykdommer?

	Mor	Far	
Kronisk obstruktiv lungesykdom	a ☐	h ☐	
Annen kronisk lungesykdom	b ☐	i ☐	
Diabetes mellitus type I	c ☐	j ☐	
Diabetes mellitus type II	d ☐	k ☐	
Reumatiske/bindevevssykdommer	e ☐	l ☐	Hvilke:_____
Inflammatorisk tarmsykdom	f ☐	m ☐	
Andre autoimmune sykdommer	g ☐	n ☐	Hvilke:_____

19.Har mor, far eller søsken hatt eller har de i dag noen av følgende sykdommer? ☐
1.Ja
2.Nei
3.Vet ikke

20.Hvis ja, hvem?

	Mor 1.Ja	Mor 2.Usikker	Far 1.Ja	Far 2.Usikker
Astma	a ☐		f ☐	
Rhinoconjuctivitt	b ☐		g ☐	
Matvareallergier	c ☐		h ☐	
Atopisk eksem	d ☐		i ☐	
Urticaria/anafylaksi	e ☐		j ☐	

21.

Helsøsken Ja, antall	Helsøsken Usikker, antall	Halvsøsken Ja, antall	Halvsøsken Usikker, antall
a ☐☐	f ☐☐	k ☐☐	p ☐☐
b ☐☐	g ☐☐	l ☐☐	q ☐☐
c ☐☐	h ☐☐	m ☐☐	r ☐☐
d ☐☐	i ☐☐	n ☐☐	s ☐☐
e ☐☐	j ☐☐	o ☐☐	t ☐☐

Sosial/Fritid

22.Har barnet gått i barnehage? ☐
1.Ja
2.Nei

23.Hvis ja, hva var barnets alder?
Fra ☐ år (a)
Til ☐ år (b)

24.Hvilken type barnehage? ☐
1.Offentlig
2.Steiner
3.Privat
4.Både privat og offentlig

25.Har barnet byttet barnehage? ☐
1.Ja
2.Nei

26.Hvis ja, antall ganger: ☐
1.En
2.To
3.Tre eller mer

27.Hvor gikk barnet i barnehage? ☐
1.Oslo
2.Annet sted
3.Oslo og annet sted

28.Hvis i Oslo, i hvilken bydel lå barnehagen? ☐☐

29Alder:
Fra ☐ år (a)
Til ☐ år (b)

Barnehagens navn: _____

Intervjuskjema Miljø og Barneastma - del 2
Hjemmemiljø

Kode ☐☐☐☐

41Har barnet bodd i samme bolig fra fødselen?
☐ 1.Ja
2.Nei

42.Hvis nei, hvor mange ganger har barnet flyttet?
☐ 1.En 2.To
3.Tre 4.>Tre

43.Har det vært egenskaper ved boligene som har gjort at barnet har flyttet? ☐ 1.Ja
2.Nei

44Hvis ja, hva var det med boligen som påvirket flyttingen?
a☐ Trangboddhet
b☐ Fukt/råteskader
c☐ Nærhet til traffikert vei
d☐ Nærhet til industriområde
e☐ Annet
Hva: _____

45.Hvor lenge har barnet bodd i nåværende bolig?
☐☐ år

46.Hvordan synes du at innemiljøet i deres nåværende bolig er?
☐ 1.Bra
2.Dårlig
3.Vet ikke

47.Hva slags boligtype bodde/bor barnet i?
Første leveår ☐ a
Nå ☐ b
1.Leilighet
2.Rekkehus/2-mannsbolig
3.Enebolig
4.Gård/småbruk
5.Annet
Hva: _____

48.Hva var/er hovedbyggematerialet i boligen?
Første leveår ☐ a 1.Tre 2.Mur Nå ☐ b

49.Hadde/har boligen flatt tak?
Første leveår ☐ a 1.Ja 2.Nei Nå ☐ b

50Hadde/har boligen takvindu?
Første leveår ☐ a 1.Ja 2.Nei Nå ☐ b

51.Hva var/er boligens byggeår?
Første leveår ☐☐☐☐ a Nå ☐☐☐☐ b

52.Var/er det vegg til vegg teppe på gulvet?

	Første leveår		Nå	
Barnets soverom	a ☐	1.Ja 2.Nei	b ☐	
Stue	c ☐		d ☐	

53.Fantes/finnes det i boligen? Hvis ja, var det i barnets soverom?
Luftfukter:
Første leveår ☐ a 1.Ja 2.Nei Nå ☐ b
Første leveår ☐ c 1.Ja 2.Nei Nå ☐ d

54.Ventilasjonsanlegg:
Første leveår a ☐ 1.Ja 2.Nei Nå b ☐

55.Luftrenser: Hvis ja, var det i barnets soverom?
Første leveår ☐ a 1.Ja 2.Nei Nå ☐ b
Første leveår ☐ c 1.Ja 2.Nei Nå ☐ d

56.Sentralstøvsuger
Første leveår ☐ a 1.Ja 2.Nei Nå ☐ b

57.Var boligen nylig malt innvendig?
Første leveår ☐ a 1.Ja 2.Nei Nå ☐ b

58.Var barnerommet nylig malt?
Første leveår ☐ a 1.Ja 2.Nei Nå ☐ b

59.Har det vært tegn til fuktskader i boligen?
Første leveår ☐ a 1.Ja 2.Nei Nå ☐ b

60.Hvor var fuktskaden?
Første leveår ☐ a 1.Barnets soverom 2.Andre rom Nå ☐ b

61.Hvis ja, hvilke?

	Første leveår		Nå		Første leveår		Nå
Mugg	a ☐	1.Ja 2.Nei	e ☐	Synlig vannlekkasje	c ☐	1.Ja 2.Nei	g ☐
Fuktflekker	b ☐		f ☐	Lukt	d ☐		h ☐

62.Var det kondens(dugg) på vinduet? Hvis ja, hvor mange cm opp fra nedre karm?
Første leveår ☐ a 1.Ja 2.Nei Nå ☐ b
Første leveår ☐☐ c Nå ☐☐ d

63Har det vært utbedret fuktskader mens barnet har bodd i boligen? ☐ a 1.Ja 2.Nei
Hvis ja, hvor gammelt var barnet? ☐☐ år b

64. Har dere noen gang hatt kjæledyr? ☐ 1.Ja 2.Nei

65. Hvis ja, hvilket/hvilke?
☐ a Katt ☐ b Hund ☐ c Kanin ☐ d Andre

66. Fantes/finnes det katt i barnets eget hjem? Første leveår a ☐ Nå b ☐ 1.Ja 2.Nei

67. Var/er barnet jevnlig i kontakt med katt? Første leveår a ☐ Nå b ☐ 1.Ja 2.Nei

68. Fantes/finnes det hund i barnets eget hjem? Første leveår a ☐ Nå b ☐ 1.Ja 2.Nei

69. Var/er barnet jevnlig i kontakt med hund? Første leveår a ☐ Nå b ☐ 1.Ja 2.Nei

70. Fantes/finnes det andre pelsdyr i barnets hjem? Første leveår a ☐ Nå b ☐ 1.Ja 2.Nei

71. Var/er barnet jevnlig i kontakt med andre pelsdyr? Første leveår a ☐ Nå b ☐ 1.Ja 2.Nei
Hvilke _____

72. Fantes/finnes det fugl i barnets eget hjem? Første leveår a ☐ Nå b ☐ 1.Ja 2.Nei

73. Var/er barnet jevnlig i kontakt med fugl? Første leveår a ☐ Nå b ☐ 1.Ja 2.Nei

74. Har familien bevisst unngått å anskaffe eller fjernet kjæledyr pga allergier i familien? ☐ 1.Ja 2.Nei

75. Forebygge allergier hos barnet? ☐ 1.Ja 2.Nei

	Første leveår	Nå
76. Hvor mange personer delte/deler barnet soverom med?	a ☐☐	b ☐☐
77. Hvor mange personer bodde/bor det i boligen?	a ☐☐	b ☐☐
78. Hvor mange rom var/er det i boligen (eksl kjøkken /bad)?	a ☐☐	b ☐☐
79. Hva var avstanden fra bolig til gjennomfartsvei? (i hundre meter)	a ☐☐	b ☐☐

80. I hvilken etg ligger/lå barnets soverom?
Første leveår a ☐ Nå b ☐
1.Kjeller/første etg uten kjeller
2.Første etg eller høyere

Kode ☐☐☐☐

81. Hadde barnet åpent soveromsvindu første leveår? Sommer a ☐ Vinter b ☐ 1.Ja 2.Nei

82. I barnets første leveår, hvor langt var det fra barnets soveromsvindu til nærmeste bjørketre? ☐
1. < 5meter
2. 5-10 meter
3. >10 meter

83. Hva slags vinduer var/er det i barnets soverom?
Første leveår a ☐ Nå b ☐
1.Flerlags vinduer
2.Enkle vinduer
3.Annet

84. Hva slags sengetøy bruker/brukte barnet?
Første leveår a ☐ Nå b ☐
1.Syntetisk
2.Fjær/dun
3.Tepper
4.Annet materiale

85. Hva slags pute brukte/bruker barnet?
Første leveår a ☐ Nå b ☐
1.Syntetisk
2.Fjær/dun
3.Bruker ikke pute

86. Hadde/har barnet middovertrekk på madrassen?
Første leveår a ☐ Nå b ☐ 1.Ja 2.Nei

87. Brukte barnet regelmessig smokk første leveår? ☐ 1.Ja 2.Nei

88. Hva slags brensel benyttet/benytter du for å lage mat?
Første leveår a ☐ Nå b ☐
1.Elektrisitet
2.Gass
3.Annet

89. Hvordan var/er hjemmet oppvarmet?

	Første leveår	Nå
Sentralvarme/elektrisitet	a☐	e☐
Innendørs ovn med gass	b☐	f☐
Innendørs ovn med olje/parafin	c☐	g☐
Innendørs ovn m ved/koks/kull	d☐	h☐

179.Hvor mange perioder har barnet fått febernedsettende pr år?

Første to leveår [ ] a (1.Aldri 2.<2 3. 3-5 4. >5)

Fra 2 års alder [ ] b

For hva: _____ For hva: _____

180.Type febernedsettende: [ ] 1.Paracetamol 2.Acetylsalicylsyre

181.Bruker/har barnet brukt andre typer medikamenter?(ikke astma/allergimedisiner) [ ] 1.Ja 2.Nei

Hvis ja,
for hva: _____

hvilket medikament: _____

i hvilken periode: _____

Atopisk sykehistorie

182.Har barnet fått påvist en eller flere allergier, ikke matvareallergier? a [ ] 1.Ja 2.Nei

Hvis ja, alder: [ ][ ] b

183.Har barnet noen gang fått diagnosen høysnue, allergisk rhinitt? a [ ] 1.Ja 2.Nei

Hvis ja, alder: [ ][ ] b

184.Har barnet noen gang hatt følgende symptomer uavhengig av forkjølelse? a [ ] 1.Ja 2.Nei

Hvis ja, hva var debut alder: b [ ][ ]

hvilke:

c☐ Rennende nese
e☐ Nesetetthet
d☐ Kløende/rennende øyne
f☐ Nysing

185.Har barnet noen gang i.l.a. de siste 12 mnd hatt følgende symptomer uavhengig av forkjølelse? a [ ] 1.Ja 2.Nei

Hvis ja, hvilke:

b☐ Rennende nese
d☐ Nesetetthet
c☐ Kløende/rennende øyne
e☐ Nysing

Hvis ja, når har barnet symptomer?

f [ ] 1.Hele året 2.Vår/sommerhalvåret 3.Høst/vinterhalvåret

186.Hva er det som utløser disse øye/nese sympt?

a☐ Vet ikke
f☐ Trepollen
b☐ Hund
g☐ Andre pollen
c☐ Katt
h☐ Temperaturforandring/ fysisk aktivitet
d☐ Andre pelsdyr
e☐ Annet

Hva: _____

Kode [ ][ ][ ][ ]

187.I hvor stor grad har disse øye/nese symptomene innvirket på de daglige aktivitetene?

[ ] 1.Ikke i det hele tatt 2.Litt 3.Moderat 4.Mye

188.Har barnet vært utredet pga øye/nese symptomene? a [ ] 1.Ja 2.Nei

Alder første gang: b [ ][ ]

Hvis ja. hvordan?

c☐ Prikktest
e☐ Sykehistorie alene
d☐ Provokasjon
f☐ Blodprøver(Spesifikk IgE)

189.Hva slags behandling har barnet fått for sine øye/nese symptomer?

a☐ Ingen behandling
b☐ Vet ikke
c☐ Lokale antihistaminer
d☐ Systemiske antihistaminer
e☐ Lokale steroider
f☐ Systemiske steroider
g☐ Natriumkromoglikat
h☐ Leukotrienantagonist
i ☐ Hyposensibilisering
j ☐ Homeopati
k☐ Annen alternativ behandling

Hva: _____

190.Har barnet hatt/har barnet i dag atopisk eksem (barneeksem)? a [ ] 1.Ja 2.Nei

Alder ved symptomstart b [ ][ ]

Hvis ja,
har barnet fortsatt atopisk eksem? c [ ] 1.Ja 2.Nei

Hvis nei,
alder ved symptomslutt: d [ ][ ]

191.Hva forverrer/forverret eksemet?

a☐ Fødemidler med melk
b☐ Fødemidler med egg
c☐ Andre fødemidler
d☐ Andre allergier
e☐ Annet - Hva: _____

63600

Intervjuskjema Miljø og Barneastma - del 2
Respirasjon

Kode ☐☐☐☐

209.Har barnet hatt tung pust, tetthet eller piping/vesing i brystet?

0-3 år: a ☐ 1.Ja 2.Nei

Etter fylte 4 år: b ☐ 1.Ikke etter fylte 4 år 2.Etter fylte 4 år

210.Har barnet hatt tørr hoste om natten uten å være forkjølet eller ha andre L VI?

a ☐ 1.Ja 2.Nei

b ☐ 3.Siste 12 mndr 4.Siste 14 dager

211.Hvor mange perioder med tung pust, tetthet eller piping/vesing i brystet har barnet hatt siden det var 6 år?

☐ 1. Ingen 2. 1-3 3. 4-12 4. mer enn 12

212.Hvor mange perioder med tung pust, tetthet eller piping/vesing i brystet har barnet hatt siste 12 måneder?

☐ 1. Ingen 2. 1-3 3. 4-12 4. mer enn 12

213.Hvor mange dager med tung pust, tetthet eller piping/vesing i brystet har barnet hatt siste 14 dagene?

☐ 1. Ingen 2. 1-3 3. 4-12 4. mer enn 12

214.Er/var det årstids- variasjon i barnets symptomer? ☐ 1.Ja 2.Nei

215.Hvis ja, hvilken/hvilke årstider er verst?

	Før skolealder	Etter skolealder
Vår	a☐	e☐
Sommer	b☐	f☐
Høst	c☐	g☐
Vinter	d☐	h☐

216.Hva er/var det som utløser/forverrer symptomene?

	1.Før	2.Nå		1.Før	2.Nå
Anstrengelse	a☐	k☐	Infeksjoner	f☐	p☐
Sigarettrøyk	b☐	l☐	Sterke lukter	g☐	q☐
Pollen	c☐	m☐	Pelsdyr	h☐	r☐
Mat/drikke	d☐	n☐	Kald luft	i☐	s☐
Tåke/rå luft	e☐	o☐	Annet,	j☐	t☐

Hva _____

217.Hvor har barnet vært undersøkt?

☐ 1.Ingen utredning 2. Almenpraktiker 3. Spesialist lungemedisin/pediatri 4. Poliklinikk ved sykehus

218.Hvor går barnet til kontroll?

☐ 1.Ingen kontroll 2.Almenpraktiker 3.Spesialist lungemedisin/pediatri 4.Poliklinikk ved sykehus

219.Hva slags undersøkelser har vært foretatt?

a☐ Ingen utredning
b☐ Flow/volum
c☐ Reversibilitetstest
d☐ Spesialistvurdering
e☐ Tredemølletest
f☐ Metacholin test

220.Har barnet noen gang fått diagnosen astma? a ☐ 1.Ja 2.Nei

Hvis ja, hvilken alder? b ☐☐ år

Hvis ja, har barnet etter din mening fortsatt astma? c ☐ 1.Ja 2.Nei

Hvis nei, alder ved symptomslutt: d ☐☐ år

221.Har lege snakket med dere om tobakksrøyking i forhold til astma? a ☐ 1.Ja 2.Nei

Hvis ja, hvem tok initiativet? b ☐ 1.Dere 2.Legen 3.Vet ikke

Savner dere informasjon om astma og røyking? c ☐ 1.Ja 2.Nei

222.Har barnet noen gang brukt medisin for luftveiene? ☐ 1.Ja 2.Nei

223.Hvis ja, kun hostesaft, Efedrin? ☐

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